AD_____

COOPERATIVE AGREEMENT NUMBER DAMD17-94-V-4039

TITLE: Validation of Rare Plant Species on Some U.S. Military Installations

PRINCIPAL INVESTIGATOR: Dr. Robert B. Shaw

CONTRACTING ORGANIZATION: Colorado State University
Center for Ecological Management of Military Lands
Fort Collins, Colorado 80523

REPORT DATE: October 1996

TYPE OF REPORT: Final

PREPARED FOR: Commander
U.S. Army Medical Research and Materiel Command
Fort Detrick, Frederick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for public release;
distribution unlimited.

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

DTIC QUALITY INSPECTED 1

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	October 1996	Final (30 Sep 94 - 29 Sep 96)

4. TITLE AND SUBTITLE
Validation of Rare Plant Species on Some U.S. Military Installations

5. FUNDING NUMBERS
DAMD17-94-V-4039

6. AUTHOR(S)
Dr. Robert B. Shaw

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Colorado State University
Center for Ecological Management of
Military Lands
Fort Collins, Colorado 80532

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Commander
U.S. Army Medical Research and Materiel Command
Fort Detrick, Frederick, Maryland 21702-5012

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

19961108 041

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200*

The effective conservation of a species depends on the availability of adequate genetic diversity within the specie's gene pool. This is especially important for rare plant species which are usually limited in numbers of individuals. In addition, the ability to reproduce successfully is critical to long term survival of a species. Genetic diversity within several rare plant species found on various U.S. military installations was evaluated, as were the requirements for germination and seedling establishment of one rare species, *Croton alabamensis*, found at Fort Hood, Texas. The species for which genetic diversity evaluations were conducted include several species of *Tetramolpoium,* species of *Silene*, and two species of *Lipochaeta* from Pohakuloa Training Area (PTA), Hawaii; *Lepidium papilliferum* from Orchard Training Center, Idaho; *Croton alabamensis* var. *texensis* from Fort Hood, Texas; and *Balduina atropurpurea* from Fort Stewart, Georgia. Genetic evaluations were based on isozyme and Random Amplified Polymorphic DNA (RAPD) analyses. The results of these evaluations will be used to aid in the development of recovery plans for each of these rare species.

14. SUBJECT TERMS	15. NUMBER OF PAGES
genetic diversity, RAPD analysis, germination	54
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

____ Where copyrighted material is quoted, permission has been obtained to use such material.

____ Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

____ Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

____ In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and use of Laboratory Animals of the Institute of Laboratory Resources, national Research Council (NIH Publication No. 86-23, Revised 1985).

____ For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

____ In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

____ In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

____ In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

R.B. Shaw 10-28-96

PI - Signature Date

(by Tracy Halward)

Table of Contents

Germination and Seedling Establishment of *Croton alabamensis* var. *texensis* at Fort Hood, Texas

Tracy Halward
Center For Ecological Management of Military Lands, Colorado State University

Introduction

Croton alabamensis E. A. Smith ex Chapman (Euphorbiaceae) was described by Farmer and Thomas (1969) as "one of the rarest shrubs in the United States." Until recently, the species was thought to be restricted to isolated populations in Tuscaloosa and Bibb Counties, Alabama (Aplet et al. 1994). In 1990, a population was discovered growing on the U.S. Army's Fort Hood, Texas by J. Cornelius, a wildlife biologist with the Fort Hood Resource Management Department. Two additional populations were later discovered by C. Sexton in the Post Oak Ridge area of Travis County, south of Fort Hood. Ginzbarg (1992) considered the Texas populations morphologically distinct enough to merit varietal status and described them as *C. alabamensis* E. A. Smith ex Chapman var. *texensis* Ginzbarg.

Croton alabamensis var. *texensis* is a relatively short-lived (20 - 30 y), monoecious shrub, typically < 3 m tall. Mature plants are multistemmed, with stems arising from a woody base at or just below the soil level. Leaves are bright green, with silvery scales above; the undersides of the leaves are covered with silvery scales dotted with conspicuous copper scales, giving the plants a distinct coppery appearance during breezy conditions. Flowers are inconspicuous and bloom in early spring from buds formed in late spring of the previous year (Ginzbarg 1992) (Figure 1).

Very little is known about the reproductive biology of either variety of *C. alabamensis*. The inflorescence of var. *texensis* is a terminal 6 - 14 flowered raceme with 1 - 6 pistillate flowers near the base and 4 - 12 staminate flowers above (Ginzbarg 1992). The degree of self-fertilization vs. out-crossing has not yet been determined. Farmer (1962) suggested that var. *alabamensis* is wind pollinated; however, in var. *texensis,* beetles have been observed feeding on pollen and pollen grains have been observed adhering to the beetles' bodies, indicating they may play a role in pollination (Steed 1993). Fruits consist of 3 (-4)-celled capsules which explosively dehisce, releasing their seeds in late May to early June. Seeds, which are approximately 7 - 8 mm long and 5 - 6 mm wide, are dark brown to almost black with white blotches and streaks, and have a yellow caruncle below a prominent white keel at the point of attachment (Ginzbarg 1992) (Figure 1). Both varieties of *C. alabamensis* typically form locally dense populations making it difficult to distinguish individual plants. Asexual reproduction through nodal rooting of prostrate branches (layering) has been observed in var. *texensis*, which adds to the difficulty of distinguishing individuals. According to Farmer (1962), no asexual reproduction had been observed among plants of var. *alabamensis*; however, Ginzbarg (1992) reported observing root layering of lower branches in some Alabama populations.

Historically, *C. alabamensis* var. *alabamensis* been reported from only 10 sites, occupying less than 100 acres (40 ha), over approximately 30 mi^2 (77.7 km^2) along the Cahaba River valley in Bibb County (7 populations) and the Warrior River valley (3 populations) in Tuscaloosa County, Alabama (Farmer and Thomas 1969). Several previously undescribed populations were recently found by A. Schotz of the Alabama Natural Heritage Program (A.

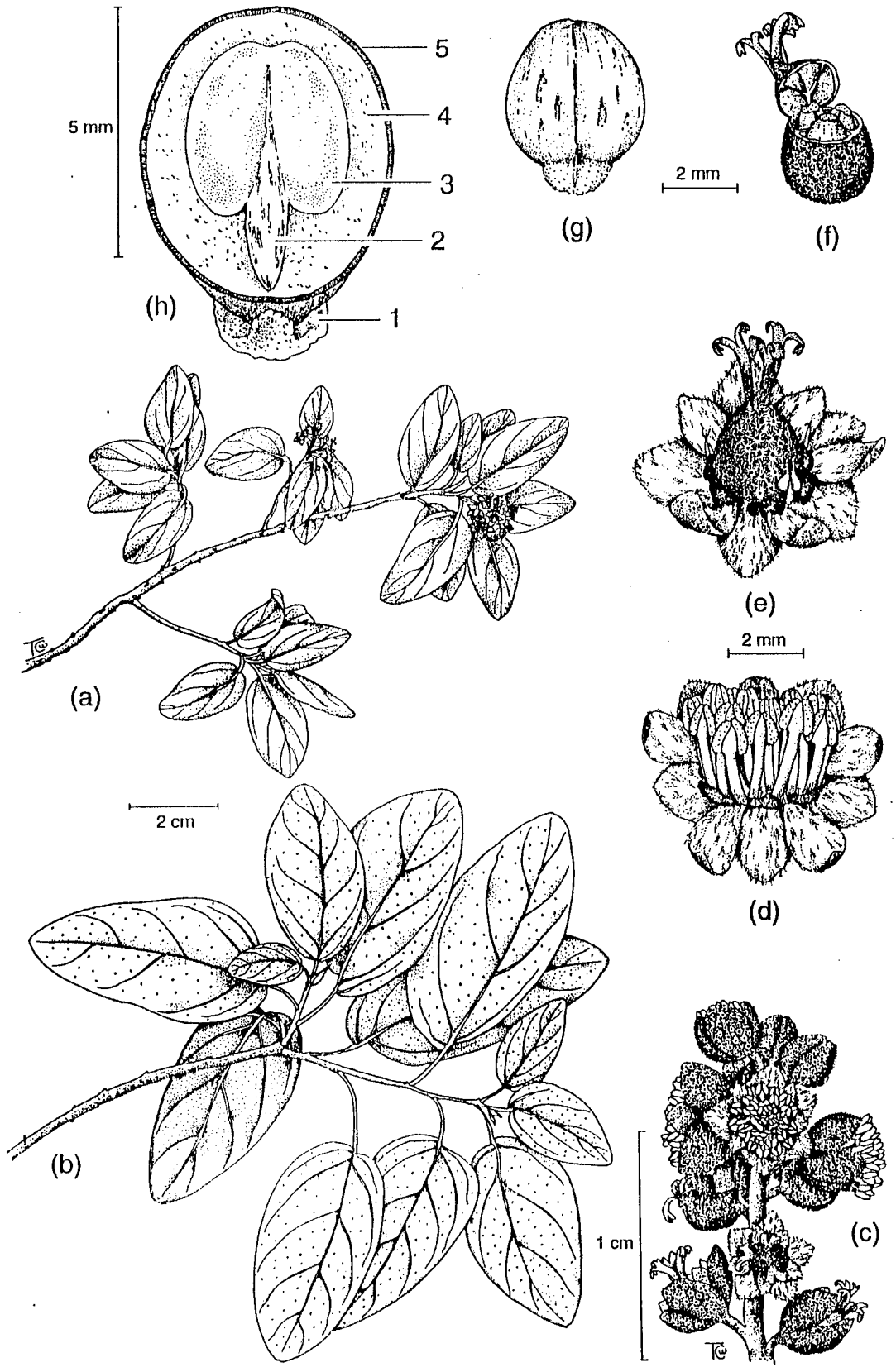

Figure 1. *Croton alabamensis* var. *texensis*: (a) stem with flowers clustered at apex of primary and secondary branches (flowers open before leaves are fully expanaded); (b) stem showing fully developed leaves; (c) androgenous raceme inflorescence; (d) male flower; (e) female flower wit rudimentary stamens; (f) 3-celled capsule; (g) seed; (h) cross section of seed: 1. caruncle, 2. embryonic radicle, 3. embryonic cotyledons, 4. endosperm, 5. seed coat

Schotz 1996, pers. comm.). The species was reported to have been collected once in 1899 in Tullahoma, Coffee County, Tennessee (Farmer and Thomas 1969); however, there have been no subsequent reports of populations from that area.

Croton alabamensis var. *texensis* has been reported only from Travis, Coryell, and Bell Counties, Texas, with most populations found along the eastern edge of the Edwards Plateau in the Owl Creek Mountains (Ginzbarg 1992). The current known distribution is limited to Fort Hood (Training Areas 2 and 3A in Coryell and Bell counties), and to a few km^2 in northwestern Travis County (Steed 1993). Croton populations typically occur on canyon bottoms and slopes in mesic ravines characterized by moderately alkaline, stony clay or clay-loam overlying Cretaceous limestone (McCaleb 1985; Werchan et al. 1974). In contrast to the Alabama populations, which are reported to grow on shallow-soiled slopes (McDaniel 1981), Aplet et al. (1994) found a significant positive association among the Texas populations between the presence of adult croton plants and areas with deep soil. They also reported no correlation between canopy gaps and presence or absence of croton, with populations observed in full sun, partial shade and densely shaded areas. However, Steed (1993) reported a correlation between canopy cover and reproductive success. Populations occurring in canopy gaps, which receive full sun most of the day and partial shade the remainder of the day, typically form dense groves and produce considerably more flowers and fruits than those found in deeply shaded areas. However, individuals found growing in full sun, with little or no shade during any part of the day, tend to be stunted and less prolific (Steed 1993).

C. alabamensis is of particular interest as it combines narrow endemism with a disjunct distribution. The Texas and Alabama populations are separated by > 1000 km and the species is not known to occur anywhere between these two regions (Figure 2). The reason(s) for the disjunction are unknown; however, several possible explanations have been proposed, including relictual disjunction occurring during periods of glaciation and relatively recent introduction of the species to Texas via long distance dispersal by birds during migration events (Ginzbarg 1992). Both varieties of *C. alabamensis* form widely dispersed but locally dense populations that appear to be in overall good health. However, the species is absent from many apparently suitable sites near existing populations. The density of local colonies suggests that the explosive dehiscence of capsules and root layering of prostrate branches are the primary forms of reproduction (Ginzbarg 1992). Both doves and quail have been observed eating croton seeds (Johnston 1959) and it is likely that the fruits/seeds are eaten by other birds as well. Intact seeds passing through a bird's digestive tract could be responsible for secondary dispersal and the establishment of new colonies (Ginzbarg 1992). Piles of empty capsules are often observed under the parent plants indicating that the fruits may also be consumed, and the seeds subsequently dispersed, by rodents.

The Fort Hood populations of *C. alabamensis* var. *texensis* are concentrated in two main canyons, "Croton Canyon" and "Sycamore Canyon," with smaller populations found at several widely dispersed locations (Figure 3). Between the two main canyons lies a third canyon ("Pear Tree Canyon") wherein croton is nearly absent, with the exception of a few individuals. The three canyons are quite similar in dominant vegetation, total overstory and understory cover, mean steepness of the canyon walls, watershed sizes, and soil depth; the only significant differences being the stream gradient and steepness of the creekbed, which are significantly

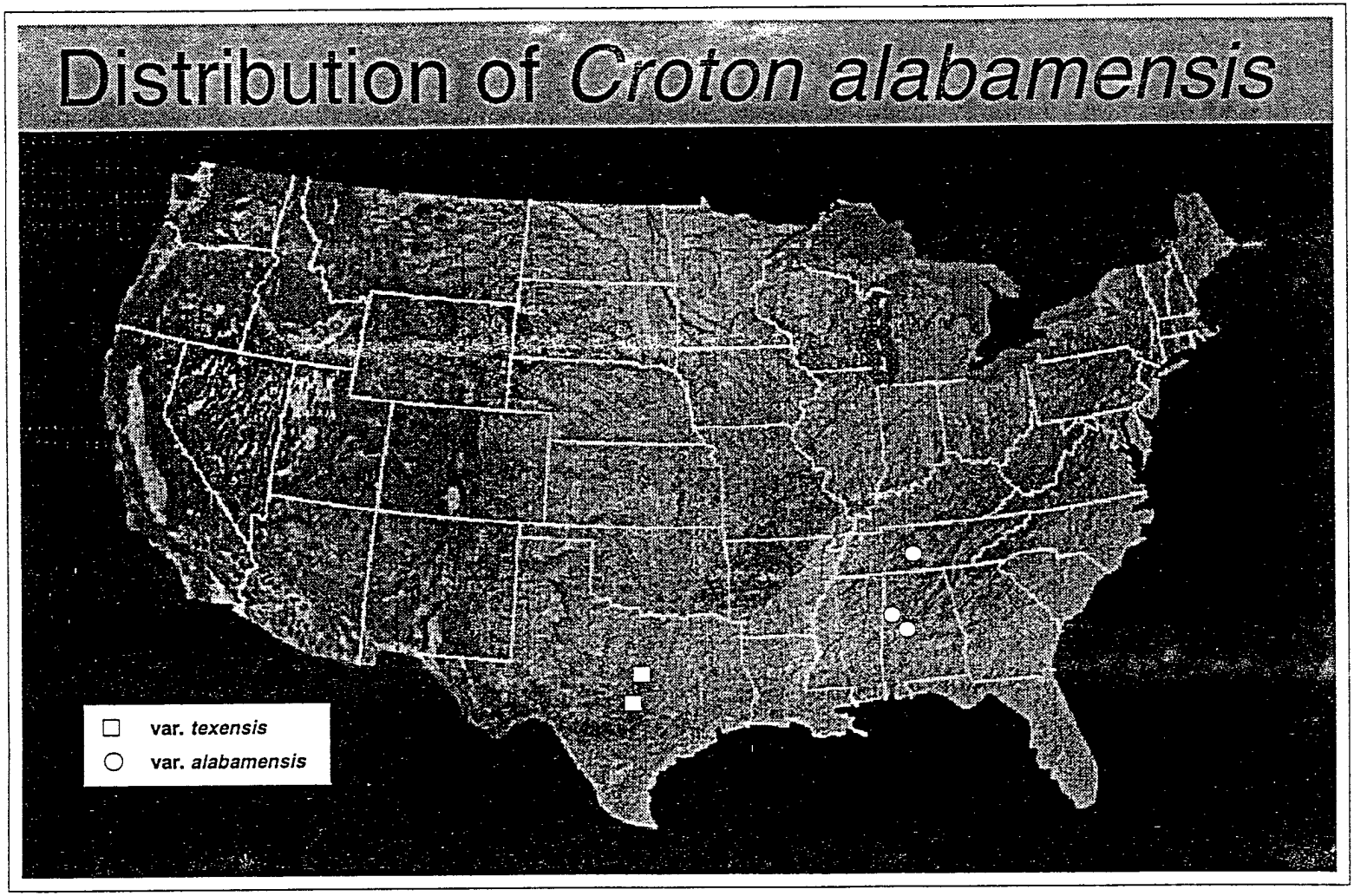

Figure 2. U.S. distribution of *Croton alabamensis*

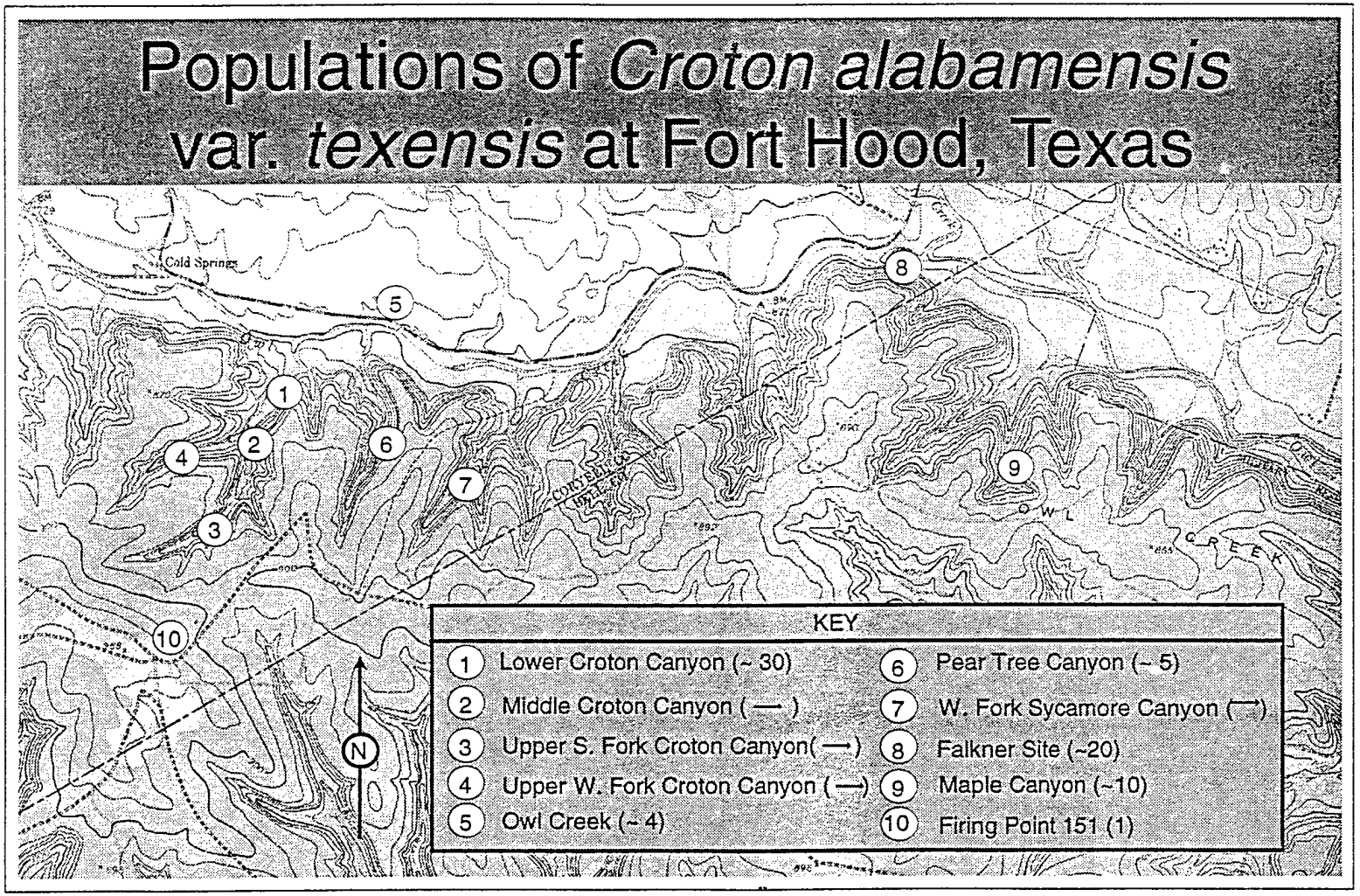

Figure 3. Distribution of populations of *Croton alabamensis* var. *texensis* at Fort Hood, Texas

greater in "Pear Tree Canyon" (Aplet et al. 1994). In Travis County, populations primarily occur in deciduous forest in mesic limestone canyons dominated by vegetation similar to that found in those canyons at Fort Hood in which croton is abundant (Ginzbarg 1992).

In addition to narrow endemism and a disjunct distribution, *C. alabamensis* is of interest for a variety of other reasons. Many *Croton* species are rich in alkaloids and terpenoids (Farnsworth et al. 1969), and several species have been utilized as sources of medicinal compounds (Webster 1993). It is quite likely that *C. alabamensis* is also rich in potentially useful compounds. On Fort Hood, many of the populations occur in woodland areas dominated by *Fraxinus texensis* (Gray) Sarg., *Quercus muhlenbergii* Engelm., *Quercus texana* Buckl. and *Juniperus ashei* Buchh., which meet the essential habitat criteria for two rare bird species, the black-capped vireo (*Vireo atricapilla*) and the golden-cheeked warbler (*Dendroica chrysoparia*) (Aplet et. al. 1994). A number of other rare plant species share the canyons occupied by croton, although most are also abundant in nearby canyons from which croton is absent. Among these rare species is another candidate for Federal listing, the orchid *Hexalectris nitida* (Steed 1993). The occurrence of several rare species in these canyons suggests the presence of unique microhabitat conditions and a rich biological history. Due to its limited distribution and potential importance, *C. alabamensis* is being considered for listing under the Endangered Species Act (ESA) of 1973, as amended. It is currently considered a "species of concern" (formerly, a category 2 candidate species).

This study was designed to determine the conditions necessary for successful germination and seedling establishment of *C. alabamensis* var. *texensis*. The results of these research efforts will be used to aid in the development of management plans for the species at Fort Hood.

Methodology

Populations Evaluated

Seeds of *C. alabamensis* var. *texensis* were collected in May 1995 from all of the major known populations at Fort Hood, Texas:

Lower Croton Canyon (population #1)
Middle Croton Canyon (population #2)
Upper South Fork Croton Canyon (population #3)
Upper West Fork Croton Canyon (population #4)
Owl Creek (population #5)
Pear Tree Canyon (population #6)
West Fork Sycamore Canyon (population #7)
Maurya's Test Site (population #8)
Maple Canyon (population #9)

Seeds were collected from multiple plants at each site, with seeds from individual plants kept separate for genetic analysis. A random bulk sample of capsules was also collected from the most prolific populations (1,2,3, and 7) for use in preliminary investigations. Capsules were transported to Colorado State University and dried in the greenhouse for one week, at which time all mature capsules had dehisced and ejected their seeds. Capsules from individual plants were kept separate, and individual drying trays were covered with mosquito netting to prevent cross contamination of containers by dehiscing seeds.

Viability Analysis

Viability analyses were conducted on 50 seeds from the bulk sample. Seed viability was determined using standard Tetrazolium (TZ) staining methodology (ISTA 1985). Briefly, after soaking seeds in ddH_20 for 48 hrs, embryos were extracted and placed in 0.1% TZ, incubated at 30°C for 4 hrs, then left at room temperature overnight.

Germination Trials

Germination trials were conducted on seeds from the bulk sample, except where noted, to preserve individual population collections for more in depth germination and genetic studies. For all germination trials, seeds were placed on top of sterile (autoclaved) blotters moistened with distilled water, set within petri dishes treated with 95% EtOH, and placed in germination chambers with an alternating temperature of 30°C (8 hr)/20°C (16 hr) and 8 hr light/16 hr dark per 24 hr period, unless otherwise indicated. Observations were made over a 50 d period.

Initial Evaluations: (1) Two samples of 20 intact seeds were plated out with no further treatment. (2) The caruncles were removed from 10 seeds by cutting with a scalpel. (3) Ten seeds were placed in a 107°C oven for 30 min in an attempt to melt the wax-like caruncles. (4) Ten seeds were soaked in ddH_20 for 48 hrs, after which the seed coats were removed. (5) Embryos were extracted from 10 seeds and the cotyledons were clipped on half of the extracted embryos, while the others were left intact. (6) Thirty seeds were soaked for 30 min in a 1.5% solution of sodium hypochlorite (dilute bleach), with constant stirring, then rinsed 6 times in ddH_20. Of these, 15 seeds were left intact and the caruncles were removed from the remaining seeds.

GA_3 Soak-1: The caruncles were removed from 30 seeds. Of these, samples of 6 seeds: (1) were given no further treatment; (2) were clipped on the non-radicle ends; (3) had the seed coats removed; (4) were cut in half longitudinally; and (5) had the embryos extracted from them. All seeds and embryos were soaked for 24 hr in 500 ppm GA_3 and subjected to the germination conditions described above.

GA_3 Soak-2: Samples of 5 seeds each: (1) left intact; (2) with the caruncles removed; and (3) with the caruncles removed and the non-radicle ends clipped were soaked for 24 hr in 500 ppm GA_3 and subjected to the germination conditions described above.

GA_3-moistened Blotters-1: Samples of 10 seeds each: (1) left intact; (2) with the caruncles removed; (3) with the caruncles removed and non-radicle ends clipped; (4) cut in half longitudinally; and (5) from which embryos were extracted, were placed on sterile blotter papers moistened with 500 ppm GA_3, and subjected to the germination conditions described above. All subsequent re-wetting of the blotters during incubation was with GA_3.

GA_3-moistened Blotters-2: Samples of 5 seeds each: (1) left intact; (2) with the caruncles removed; and (3) with the caruncles removed and non-radicle ends clipped, were placed on sterile blotter papers moistened with 500 ppm GA_3, and subjected to the germination conditions described above. All subsequent re-wetting of the blotters during incubation was with GA_3.

KNO_3 and HCl Treatments: For each treatment, samples of 10 seeds each: (1) left intact; (2) from which the caruncles were removed; and (3) with the caruncles removed and the non-radicle ends clipped, were soaked in either 0.2% KNO_3 or 0.2N HCl for 24 hrs and subjected to the germination conditions described above.

Dark Treatment: Samples of 10 seeds each: (1) left intact; (2) with the caruncles removed; (3) with the caruncles removed and the non-radicle ends clipped; (4) cut in half longitudinally; and (5) from which the embryos were extracted, were subjected to the germination conditions described above, except that petri dishes were placed in a sealed box inside three black plastic bags to prevent exposure to light. Evaluations were conducted in a photographic darkroom using a green-wavelength safelight. After 12 d in darkness, petri dishes were removed from the box and returned to the germinator.

Boiling: Ten intact seeds and 10 seeds from which the caruncles were removed were boiled for five minutes, cooled to room temperature, and subjected to the germination conditions described above.

Stratification-1: Thirty intact seeds and 30 seeds from which the caruncles were removed were placed on moistened blotter paper in covered petri dishes and incubated at 4°C for varying lengths of time. Samples of 10 intact seeds and 10 seeds with the caruncles removed were plated out in petri dishes following 10 d, 2 wk, and 3 wk of stratification, and subjected to the germination conditions described above.

Stratification-2: Samples of 5 intact seeds, 5 seeds with the caruncles removed, and 5 seeds with the caruncles removed plus the non-radicle ends clipped were stratified at 4°C for 4, 6, or 8 wks and subjected to the germination conditions described above. Germination response was compared to a similar series of non-stratified seeds.

Stratification-3: Three hundred seeds (70 seeds each from populations 3 and 4, and 80 seeds each from populations 7 and 8) were stratified for 8 weeks at 4°C, and then planted in the greenhouse in dibble tubes containing a commercial, soilless potting medium. Germination rate and percent germination for each population were compared to an equal number of seeds planted directly in the greenhouse with no previous stratification.

Optimal Length of Stratification Period: Seeds from the bulk sample were divided into two lots of 70; one lot was surface sterilized and the other lot was left untreated. The surface sterilized seeds were soaked in 2% sodium hypochlorite for 5 min. followed by 6 washes with ddH_2O. Ten seeds from each lot were planted in the greenhouse following 0, 2, 4, 6, 8, 10, and 12 weeks of stratification at 4°C. Germination rate, percent germination, and incidence of microbial infection of the seed at time of planting were recorded.

Greenhouse Seeding-1: Samples of 10 seeds each: (1) left intact; (2) with the caruncles removed; and (3) with the caruncles removed and the non-radicle ends clipped, were planted in pots containing a commercial, soilless potting medium and placed in the greenhouse. In addition, 5 intact seeds and 5 seeds from which the caruncles were removed were planted in pots containing sterilized sand and placed in the greenhouse.

Greenhouse Seeding-2: Samples of 5 seeds each were exposed to the following treatment combinations and subsequently planted in commercial, soilless potting medium in the greenhouse: (1) seeds left intact--a) with no further treatment, b) stratified at 4°C for 4 wk, c) stratified at 4°C for 8 wk, and d) soaked in ddH_20 at room temperature for 1 wk; (2) caruncle removed from seed--a) with no further treatment, b) stratified at 4°C for 4 wk, c) stratified at 4°C for 8 wk, and d) soaked in ddH_20 at room temperature for 1 wk; and (3) caruncle removed from seed plus non-radicle end clipped--a) with no further treatment, b) stratified at 4°C for 4 wk, c) stratified at 4°C for 8 wk, and d) soaked in ddH_20 at room temperature for 1 wk.

After-ripened Seeds: The caruncles were removed from 4 after-ripened seeds (harvested in 1992) which had been stored at room temperature, and the seeds were subjected to the germination conditions described above. These were the only after-ripened seeds available at the time the study was conducted.

Seedling Establishment

Seeds and "transplants" from the above germination trials were planted in dibble tubes filled with a commercial, soilless potting medium and maintained in the greenhouse until the appropriate time for transplanting to Fort Hood. After emergence, 6 random seedlings were carefully removed from the dibble tubes, with minimal disturbance to the seedling, to observe root development. Following observation, seedlings were carefully replaced in the dibble tubes.

Outplanting Trials

Fort Hood experienced severe drought conditions from November 1995 until mid-June 1996, when the area received a small amount of rainfall. However, it remained abnormally dry throughout the summer and transplanted seedlings were not likely to survive in the field under these adverse conditions. Therefore, the Outplanting Trials were postponed until spring of 1997 when, barring continuation of the drought, environmental conditions would be more favorable for successful seedling establishment*. Seedlings will be planted at Fort Hood within two canyons where croton occurs, as well as two canyons where croton is absent. Seedlings will be tagged to allow for future monitoring of seedling establishment. In addition, seeds will be planted in marked plots near the transplanted seedlings at each outplanting site. Directly seeded and transplanted individuals will be monitored periodically for 3 years to determine seedling survival rates and to evaluate population health. Soil samples will be taken from each site into which individuals are transplanted and analyzed for pH, soil type/texture, and nutrient content.

*NOTE: substantial rainfall occurred in the Fort Hood area in late September and early October; thus, drought conditions are improving and transplanting should be able to proceed successfully in spring 1997.

Evaluation of Fire Severity in "Croton Canyon"

In late February 1996, a 15,000 acre fire at Fort Hood burned through much of the largest *C. alabamensis* var. *texensis* populations ("Croton Canyon"). In early March, with the aid of a fire ecologist (Frankie Romero; Colorado State University), relative fire intensities were mapped throughout "Croton Canyon". Pre-burn fuel loads were estimated from similar habitat that was not affected by the fire. The fire severity analysis consisted of three parts: 1) an on-site fire severity analysis, 2) a series of fire behavior computer-simulations, and 3) interviews with eye-witnesses. For the first segment of the analysis, an on-site fire severity inventory was completed 3 weeks after the burn using the methods described by Ryan and Noste (1984). No rainfall had been recorded between the time of the fire and the analysis, thus, very little disturbance was evident at the time the analysis was conducted.

Figure 4 illustrates the layout of the basic fire severity plot. The two largest croton populations within "Croton Canyon" were located and two transect lines were established, one

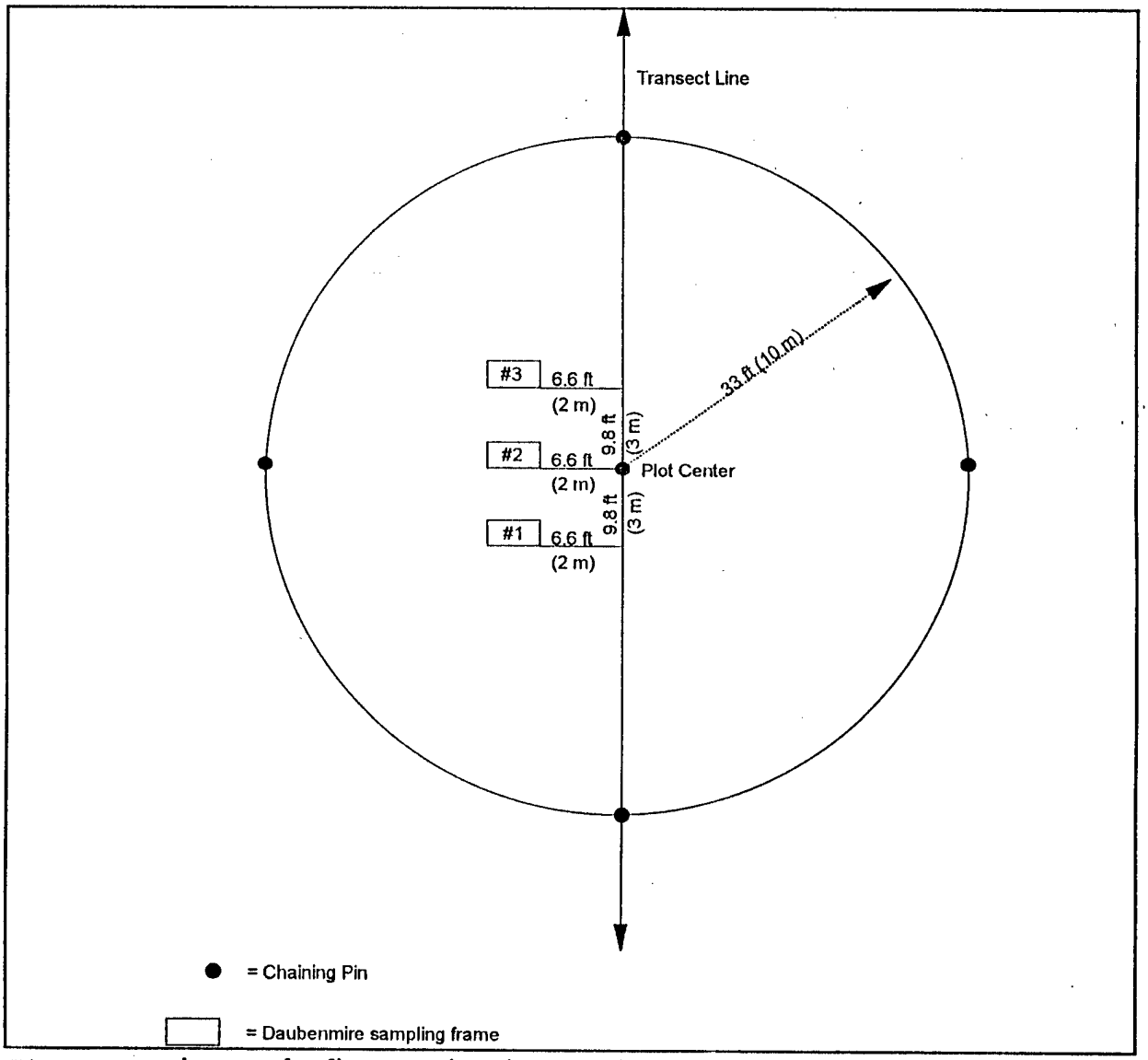

Figure 4. Layout for fire severity plots used to estimate fire severity within Croton Canyon, Fort Hood, Texas.

through each population (Figure 5). Sixteen plots were located at set intervals along these two transect lines. All plots were spaced 2 chains (1 chain = 66 ft) apart except for plots 2 and 3. These two plots were placed 1 chain apart to obtain 2 samples within the largest croton population.

The downward heat pulse was determined from three char sub-plots (Daubenmire frames). For consistency, the sub-plots were offset in the same direction (e.g., north of an east-west transect line). If the frame landed on an area with greater than 50% rock or where trampling was evident, it was moved one frame-length further away from the transect line. This action was repeated as necessary to obtain samples of undisturbed char. Degree of charring was determined using the visual characteristics from Ryan and Noste (1984), and each of the three sub-plots was classified as either Unburned, Lightly charred, Moderately charred, or Deeply charred. The entire plot was given a classification according to the majority classification of the three sub-plots.

The upward heat pulse was determined from the scorch height evidence found on the remnant trees within the 66 ft diameter plot. Scorch height is defined as the height at which plant material is destroyed by heat, thus, the height live leaf and branch material is found on a burned tree. The scorch height was translated into 1 of the 5 flame length classes as defined by Ryan and Noste (1984). These classes are shown in Table 1.

In cases where a single flame length class was not obvious, the plot was ocularly divided into quadrants and the flame length class found in the majority of quadrants was recorded. If equivalent, the average flame length class of all 4 quadrants was recorded. Average tree height and percent crown scorch were also recorded, as was an inventory of remnant stems identified by species and diameter class. If all branch and leaf material in the plot was completely consumed, it was noted and the height of the tallest tree was recorded as the scorch height for the plot. Final flame length classes for these plots were obtained from the fire behavior simulations.

Table 1. Flame length classes (Ryan and Noste 1984). * The range of crown scorch is based on Van Wagner's (1973) equation 10, assuming the flame length range for the class, 77° F, no wind, and no slope.

Flame Length Class	Flame Length Range (feet)	Corresponding Crown Scorch Height* (feet)
1	0 - 2	0 - 9
2	2 - 4	9 - 24
3	4 - 8	24 - 64
4	8 - 12	64 - 116
5	> 12	> 116

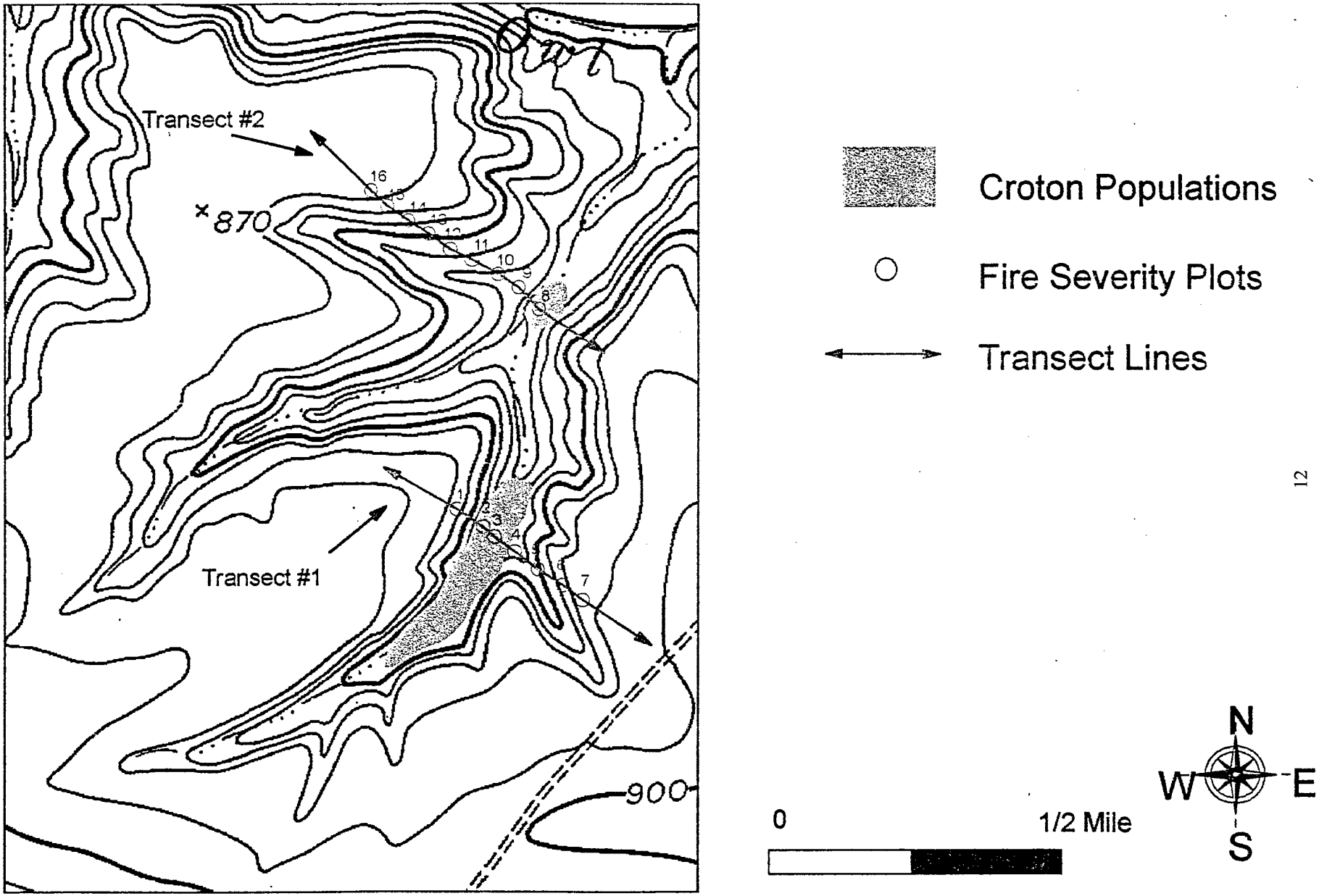

Figure 5. Locations of croton populations, fire severity transects, and fire severity plots within Croton Canyon.

The overall fire severity for each plot was determined from the combination of the upward and downward pulses using the two-dimensional fire severity matrix of Ryan and Noste (1984).

The second portion of the analysis consisted of a series of fire behavior simulations. To accomplish this, several vegetative inventories were conducted in a nearby unburned area identified by local natural resource managers as representative of the terrain and vegetation found in "Croton Canyon" (B.R. Jones, J. Cornelius, D. Herbert, T. Buchanan, Fort Hood, TX 1996, pers. comm.). These inventories were used to create custom fuel models for use in the fire behavior simulations. The methods described by Brown et al. (1982) were used to conduct the vegetative inventories as well as dead-and-down woody fuel inventories. The TESTMODEL module of the BEHAVE fire behavior prediction system was used to create and test the two custom fuel models for the fire behavior simulations (Burgan and Rothermel 1984). On-base weather stations provided the 2:00 pm weather conditions present the day the fire passed through "Croton Canyon", and these were used for the simulations. Fuel-stick moistures were also collected for the days of the fire and these were used to initialize the fuel moisture values in the fire behavior simulations. "Croton Canyon" runs in a northeast to southwest direction, thus, the fire behavior simulations were conducted for east-facing and west-facing slopes.

In the last phase of the analysis, eye-witness accounts were obtained to serve as verification for the preceding analyses. Three employees from the Office of Natural Resources at Fort Hood who were involved in the fire suppression effort were interviewed (B.R. Jones, J. Cornelius and, T. Buchanan 1996, pers. comm.). Interviews were conducted in the field to better facilitate the descriptions of the fire's behavior characteristics. Information was obtained on flame length, rate of spread, method of propagation (independent crown fire, surface fire, etc.) and characteristics of the smoke column (towering, bent, blowing, etc.) at various times and at particular locations. In addition to the eye-witness accounts, several video tape segments from local news stations were reviewed. The video segments were taken during the height of fire activity, as well as during the mop-up stages of the fire suppression effort. This provided a complete view of the range of fire behavior experienced.

From the above analyses, a table of fire severity ratings classified by aspect, slope, and fuel type was created. In addition, a map of "Croton Canyon" was created which shows the spatial distribution of the zones of fire severity found throughout the canyon.

Post-fire regeneration will be evaluated for both the croton populations and the surrounding community. Vegetation plots will be established and monitored annually, beginning 1 year post-fire.

Results

Seed Collection

The number of fruits collected varied considerably among sites due to wide variation in fruiting among populations, ranging from 1 for population 9 to 159 for population 7. In general, fruits were more abundant on plants growing in open to semi-open sites than on plants growing under dense canopy cover.

Viability Analysis

Most of the extracted embryos stained normally, except in those areas that had sustained damage during the extraction process, with > 95% viability observed among the seeds evaluated.

Germination Trials

Initial Evaluations: (1) Attempts at germination of seeds with the seed coats intact resulted in 100% of the seeds succumbing to fungal infection within 48 hrs. Fungal growth typically began at the caruncle, eventually covering the entire seed. All of these individuals were discarded after 1 wk of incubation. (2) All seeds from which the caruncles were removed by cutting developed fungal infection within 48 hrs to 1 week and were discarded after 2 weeks of incubation. (3) All intact seeds incubated at 107°C for 30 min developed fungal infections; however, onset was delayed to between 1 and 2 wk. These individuals were also discarded. (4) None of the individuals from which the seed coats were removed germinated or were infected with fungus when the trial was terminated after 50 d of incubation. (5) In all of the extracted embryos, the cotyledon next to the blotter turned green within 5 d. When inverted, the formerly upper cotyledons turned green within 2 wk. The cotyledons expanded and appeared to be developing normally; however, no radicle development was observed after 50 d. None of the extracted embryos developed microbial infections. (6) Treatment with sodium hypochlorite reduced fungal infection by approximately 70 and 80% among intact seeds and seeds with the caruncles removed, respectively, and onset of infection was delayed to between 1 and 2 wk. However, none of the treated seeds germinated during the 50 d observation period (Table 2).

GA_3 Soak-1: All seeds that were soaked for 24 hr in 500 ppm GA_3 showed extensive damage, regardless of pre-treatment, with all exposed regions turning brown and necrotic. All seeds from which the caruncles were removed and with no further treatment, developed bacterial infections within 2 wk; none had germinated after 50 d. Among seeds in which the non-radicle ends were clipped in addition to removing the caruncles, 50% developed bacterial infections; none had germinated after 50 d. All seeds from which the seed coats were removed became severely necrotic and failed to germinate, but showed no signs of microbial contamination. After 1 wk in the germinator, a radicle emerged from 1 of the seeds that had been cut in half longitudinally; after 50 d, no others showed signs of germination or microbial contamination. The seed from which the radicle emerged subsequently developed a bacterial infection. The seed coat deteriorated into a thin shell and eventually ruptured, allowing the cotyledons to emerge. The "seedling" was transferred to a pot containing a commercial, soilless potting medium and placed in the greenhouse. The "seedling" failed to develop any further and died within 1 wk of transplanting. All extracted embryos became severely necrotic, the cotyledons failed to turn green, and no further growth or development was observed during the 50 d observation period. None of the extracted embryos developed microbial infections (Table 2).

GA_3 Soak-2: Among intact seeds, all individuals developed fungal infections and none showed signs of germination during the 50 d observation period. Among seeds from which the caruncle had been removed, 1 individual developed a fungal infection and 1 became infected with bacteria. This latter individual subsequently germinated, following rupture of the seedcoat, and was transplanted into a pot containing commercial, soilless potting medium and placed in the greenhouse. Among seeds in which the non-radicle end was clipped in addition to the removal

of the caruncle, all individuals developed fungal contamination and 3 individuals also became infected with bacteria; however, none showed signs of germination at the end of the 50 d observation period (Table 2).

GA_3-moistened Blotters-1: For all seeds evaluated, regardless of pre-treatment, areas in contact with GA_3-moistened blotters turned brown and necrotic. Seven of the 10 intact seeds developed fungal infections and none germinated after 50 d. Among seeds with the caruncles removed, 1 developed fungal infection and 2 became infected with bacteria; none germinated after 50 d. Of the seeds in which the non-radicle ends were clipped in addition to removal of the caruncles, 7 showed no signs of contamination or germination; 2 developed fungal infections and failed to germinate; and 1 became infected with bacteria. After approximately 1 week, a radicle emerged from the bacterial-infected individual, and the seed coat subsequently deteriorated exposing the cotyledons. The "seedling" was transplanted into commercial, soilless potting medium in the greenhouse, where it subsequently died. Among seeds cut in half longitudinally, a radicle emerged from 1 seed; however, it was weak and necrotic, and no further development was observed. Of the remaining 9 seeds which had been cut in half longitudinally, 2 became infected with bacteria and 1 developed a fungal infection; none of these showed signs of germination during the 50 d observation period. All extracted embryos exposed to GA_3-moistened blotters turned green within 2 wk; however, no radicle development occurred during the 50 d observation period and none developed microbial infections (Table 2).

GA_3-Moistened Blotters-2: All individuals, regardless of pre-treatment developed fungal infections within 1 wk of incubation in the germinator. Two seeds (1 intact seed and 1 seed from which the caruncle was removed and the non-radicle end was clipped) developed bacterial infections, after which radicles emerged. However, the radicles on both individuals were deformed and necrotic, and the cotyledons failed to emerge (Table 2).

KNO_3 Treatment: All intact seeds, and 3 seeds from which the caruncles were removed developed fungal infections within 1 wk. Two seeds in which the non-radicle ends were clipped in addition to removal of the caruncles also succumbed to fungal infection. No other development or contamination was observed after 50 d (Table 2).

HCl Treatment: Regardless of pre-treatment, all seeds developed fungal patches on the seed coats within 1 wk. The fungus was of a different type than observed during previous trials, forming green patches on the seed coats without affecting the caruncles. Within 2 wk of incubation in the germinator, all intact seeds also developed the typical fungal infection initiated in the caruncle region. None of the seeds showed signs of germination after 50 d (Table 2).

Dark Treatment: When seeds were removed from the box after 12 d of darkness, no signs of germination were apparent, regardless of pre-treatment. One of the intact seeds had become infected with fungus; no other microbial contamination was present on any of the seeds, regardless of pre-treatment. Extracted embryos were white, with no growth or contamination. Upon exposure to light, all intact seeds developed fungal infections within 48 hr. Among seeds from which the caruncles were removed, either clipped or unclipped, none developed microbial infections after 50 d. Among seeds cut in half longitudinally, 1 developed a fungal infection and 2 developed bacterial infections. None of the dark-exposed seeds showed signs of germination after 50 d. Three of the extracted embryos turned green within 1 wk of exposure to light, while

the others remained white. Radicles of all extracted embryos became necrotic and failed to develop. None of the extracted embryos developed microbial infections (Table 2).

Boiling: None of the seeds subjected to boiling developed microbial infections, and none showed signs of germination at the end of the 50 d observation period (Table 2).

Stratification-1: Fifty percent of intact seeds developed fungal infections within 1 wk, and 100% became infected within 2 wk during incubation at 4°C. None of the seeds from which the caruncles were removed succumbed to infection during incubation at 4°C. All seeds, regardless of treatment, developed fungal infections within 1 wk of being transferred to the germinator, regardless of the length of the stratification period to which they were exposed (10d, 2 wk, or 3 wk). A radicle emerged from 1 intact seed that had been stratified for 3 wk; however, no further development was observed at the end of 50 d (Table 2).

Stratification-2: All of the intact seeds developed fungal infections within 1 wk in the germinator. A radicle emerged from 1 intact seed, which had been stratified for 4 wk; however, the radicle became necrotic and no further development was observed. No other germination was observed among intact seeds. Among seeds from which the caruncle was removed, the control seeds all developed fungal infections, and no other contamination or germination was observed. Among seeds in which the non-radicle end was clipped in addition to removal of the caruncle, all control seeds developed fungal infections, as did 1 seed stratified for 4 wk and 2 seeds stratified for 6 wk. No other contamination and no signs of germination were observed (Table 2).

Stratification-3: Only 4 of the control seeds (3 from population 8 and 1 from population 3) germinated. Of the seeds which had been stratified for 8 wk, germination occurred in 1 seed from population 7, 2 seeds from population 4, 9 seeds from population 3, and 13 seeds from population 8. The degree of microbial contamination at planting was not recorded (Table 2).

Optimal Length of Stratification Period: Most (97%) of the untreated seeds developed fungal infections during stratification. Among the seeds treated with 2.0% sodium hypochlorite, the degree of fungal contamination increased with increasing length of stratification, with 4 out of 10 seeds infected after 2 wk and all 10 seeds infected after 10 and 12 wk of stratification. Germination percentages were low, regardless of treatment or the length of stratification period. Among non-treated seeds, germination occurred in 1 control (non-stratified) seed, 2 seeds which had been stratified for 6 wk, 2 seeds which had been stratified for 10 wk, and 4 seeds which had been stratified for 12 wk. Among treated seeds, germination occurred in 2 seeds stratified for 6 wk, 1 seed stratified for 10 wk, and 3 seeds stratified for 12 wk. The seed coats had split open on 2 of the treated individuals stratified for 12 wk. The treated individuals that germinated after 6 wk of stratification showed no signs of fungal contamination at planting, whereas, those that germinated after 10 and 12 wk of stratification showed slight and moderate levels of infection, respectively, at time of planting (Table 2).

Greenhouse Seeding-1: None of the seeds planted in the greenhouse germinated after 50 d, regardless of treatment or planting medium (Table 2).

Greenhouse Seeding-2: Among intact seeds, germination occurred in 3 seeds stratified for 8 wk and 2 seeds soaked for 1 wk in ddH_20. Among seeds from which the caruncle was removed, germination occurred in 2 control seeds, 2 seeds stratified for 4 wk, 3 seeds stratified for 8 wk, and 1 seed soaked for 1 wk in ddH_20. Among seeds from which the caruncle was

removed and the non-radicle end was clipped, germination occurred in 1 control seed and 2 seeds stratified for 8 wk. The incidence of microbial contamination at planting was not recorded (Table 2).

After-ripened Seeds: After 50 d in the germinator, none of the after-ripened seeds had germinated. Three of the 4 had become slightly contaminated with fungal growth of a different type than noted on any of the freshly harvested seeds evaluated. Embryos were extracted from all 4 seeds. One of the embryos, along with the endosperm, was rotten; however, the other 3 embryos were tested for viability. All 3 of these embryos stained normally, indicating the seeds were viable despite their failure to germinate (Table 2).

Summary of Germination Results

A total of 1,319 seeds were evaluated for germination response, of which only 68 individuals (5.2%) germinated, regardless of treatment (Table 3). Of the 659 seeds for which data were collected on microbial infection, 351 individuals (53.3%) developed fungal infections, 13 (2.0%) developed bacterial infections, and 10 (1.5%) became infected with both fungus and bacteria. Twenty-one of the 23 individuals that developed bacterial infections had been treated with GA_3, while the other 2 had been exposed to 12 d of darkness during imbibition (Table 3). Among seeds evaluated for microbial contamination, 78% of intact seeds and 41% of seeds from which the caruncle was removed (with or without clipping of the non-radicle end) developed fungal infections (Table 3). None of the intact seeds developed bacterial infections, while approximately 18% of the seeds from which the caruncle was removed became infected with bacteria (Table 3). Twenty-three (3.5%) of the 659 individuals evaluated for microbial contamination germinated. Among the germinants, 14 were from seeds infected with fungal contamination, 1 was from a seed infected with bacterial contamination, and 5 were from seeds which had developed both fungal and bacterial infections prior to germination. Thus,

Table 2. Results of *C. alabamensis* var. *texensis* Germination Trials

Seed Pre-treatment	Seed Treatment	# Seeds[1]	# Fungus[2]	# Bact.[3]	# Germ.[4]	Seedling?[5]
Initial Evaluations						
none	none	40	40	0	0	na[6]
caruncle removed	none	10	10	0	0	na
none	107°C for 30 min.	10	10	0	0	na
seedcoat removed	none	10	0	0	0	na
embryo extracted	none	5	0	0	0	na
embryo extracted	cotyledons clipped	5	0	0	0	na
none	1.5% sodium hypochlorite	15	5	0	0	na
caruncle removed	1.5% sodium hypochlorite	15	3	0	0	na
GA_3 Soak-1						
caruncle removed	GA_3 soak (500 ppm)	6	0	6	0	na
caruncle removed + non-radicle end clipped	"	6	0	3	0	na
caruncle and seed coat removed	"	6	0	0	0	na
caruncle removed + cut in half longitudinally	"	6	0	1	1	1
embryo extracted	"	6	0	0	0	na
GA_3 Soak-2						
none	GA_3 soak (500 ppm)	5	5	0	0	na
caruncle removed	"	5	1	1	1	1
caruncle removed + non-radicle end clipped	"	5	5	3	0	na
GA_3-Moistened Blotters-1						
none	GA_3-moistened blotters	10	7	0	0	na
caruncle removed	"	10	1	2	0	na
caruncle removed + non-radicle end clipped	"	10	2	1	1	1
caruncle removed + cut in half longitudinally	"	10	1	2	1	0
embryo extracted	"	10	0	0	0	na
GA_3-Moistened Blotters-2						
none	GA_3-moistened blotters	5	5	1	1	0
caruncle removed	"	5	5	0	0	na
caruncle removed + non-radicle end clipped	"	5	5	1	1	0
KNO_3 Treatment						
none	KNO_3 soak (0.2%)	10	10	0	0	na
caruncle removed	"	10	3	0	0	na
caruncle removed + non-radicle end clipped	"	10	2	0	0	na
HCl Treatment						
none	HCl soak (0.2N)	10	10	0	0	na
caruncle removed	"	10	10	0	0	na
caruncle removed + non-radicle end clipped	"	10	10	0	0	na
Dark Treatment						
none	12 d darkness	10	10	0	0	na
caruncle removed	"	10	0	0	0	na
caruncle removed + non-radicle end clipped	"	10	0	0	0	na
caruncle removed + cut in half longitudinally	"	10	1	2	0	na
embryo extracted	"	10	0	0	0	na
Boiling						
none	boiled for 5 min.	10	0	0	0	na
caruncle removed	"	10	0	0	0	na

Seed Pre-treatment	Seed Treatment	# Seeds[1]	# Fungus[2]	# Bact.[3]	# Germ.[4]	Seedling?[5]
Stratification-1						
none	4°C for 10d	10	10	0	0	na
none	4°C for 2 wk	10	10	0	0	na
none	4°C for 3 wk	10	10	0	1	0
caruncle removed	4°C for 10d	10	10	0	0	na
caruncle removed	4°C for 2 wk	10	10	0	0	na
caruncle removed	4°C for 3 wk	10	10	0	0	na
Stratification-2						
none	none	5	5	0	0	na[6]
caruncle removed	none	5	5	0	0	na
caruncle removed + non-radicle end clipped	none	5	5	0	0	na
none	4°C for 4 wk	5	5	0	1	0
caruncle removed	4°C for 4 wk	5	0	0	0	na
caruncle removed + non-radicle end clipped	4°C for 4 wk	5	1	0	0	na
none	4°C for 6 wk	5	5	0	0	na
caruncle removed	4°C for 6 wk	5	0	0	0	na
caruncle removed + non-radicle end clipped	4°C for 6 wk	5	2	0	0	na
none	4°C for 8 wk	5	5	0	0	na
caruncle removed	4°C for 8 wk	5	0	0	0	na
caruncle removed + non-radicle end clipped	4°C for 8 wk	5	0	0	0	na
Stratification-3						
none	none	300	na	na	4	4
none	4°C for 8 wk	300	nr[7]	nr	25	25
Optimal Length of Stratification Period						
none	none	10	0	0	1	1
none	4°C for 2 wk	10	8	0	0	na
none	4°C for 4 wk	10	10	0	0	na
none	4°C for 6 wk	10	10	0	2	2
none	4°C for 8 wk	10	10	0	0	na
none	4°C for 10 wk	10	10	0	2	2
none	4°C for 12 wk	10	10	0	4	4
2.0% sodium hypochlorite	none	10	0	0	0	0
2.0% sodium hypochlorite	4°C for 2 wk	10	4	0	0	na
2.0% sodium hypochlorite	4°C for 4 wk	10	7	0	0	na
2.0% sodium hypochlorite	4°C for 6 wk	10	6	0	2	2
2.0% sodium hypochlorite	4°C for 8 wk	10	9	0	0	na
2.0% sodium hypochlorite	4°C for 10 wk	10	10	0	1	1
2.0% sodium hypochlorite	4°C for 12 wk	10	10	0	3	3
Greenhouse Seeding-1						
none	greenhouse-soilless medium	10	0	0	0	na
caruncle removed	"	10	0	0	0	na
caruncle removed + non-radicle end clipped	"	10	0	0	0	na
none	greenhouse--sterile sand	5	0	0	0	na
caruncle removed	greenhouse--sterile sand	5	0	0	0	na
caruncle removed + non-radicle end clipped	greenhouse--sterile sand	5	0	0	0	na

Seed Pre-treatment	Seed Treatment	# Seeds[1]	# Fungus[2]	# Bact.[3]	# Germ.[4]	Seedling?[5]
Greenhouse Seeding-2						
none	none	5	nr	nr	0	0
none	4°C for 4 wk	5	nr	nr	0	0
none	4°C for 8 wk	5	nr	nr	3	3
none	soaked in ddH_20 1 wk	5	nr	nr	2	2
caruncle removed	none	5	nr	nr	2	2
caruncle removed	4°C for 4 wk	5	nr	nr	2	2
caruncle removed	4°C for 8 wk	5	nr	nr	3	3
caruncle removed	soaked in ddH20 1 wk	5	nr	nr	1	1
caruncle removed + non-radicle end clipped	none	5	nr	nr	1	1
caruncle removed + non-radicle end clipped	4°C for 4 wk	5	nr	nr	0	0
caruncle removed + non-radicle end clipped	4°C for 8 wk	5	nr	nr	2	2
caruncle removed + non-radicle end clipped	soaked in ddH_20 1 wk	5	nr	nr	0	0
After-ripened Seeds						
caruncle removed	after-ripened naturally	4	3	0	0	na

[1] # Seeds = number of seeds evaluated for that trial; [2] # Fungus = number of individuals infected with fungal contamination; [3] # Bact. = number of individuals infected with bacterial contamination; [4] # Germ. = number of seeds that germinated; [5] Seedling? = indicated whether or not a germinant became established as a seedling; [6] na = not applicable; [7] nr = not recorded

approximately 87% of the germinants from seeds evaluated for microbial infection were contaminated prior to germination. In other words, approximately 4% of the fungal-infected seeds, 8% of the bacterial-infected seeds, and 50% of the seeds with both fungal and bacterial contamination germinated. (Table 3). By comparison, among seeds evaluated for microbial infection, germination occurred in only 3 (< 0.5%) non-contaminated seeds.

Among all intact seeds (both those evaluated for microbial contamination and those not evaluated for contamination), germination occurred in 52 of 950 individuals (5.5%); while among seeds from which the caruncle was removed (including both individuals with and without the non-radicle end clipped in addition to removal of the caruncle), 14 of 291 individuals (4.8%) germinated (Table 3).

Stratification at 4°C slightly enhanced germination. Percent germination generally increased with increasing length of the stratification period, with approximately 6% germination among individuals stratified for 4 wk and 35% germination among individuals stratified for 12 wk (Table 3).

Table 3. Summary of Results for *C. alabamensis* var. *texensis* Germination Trials

	total # seeds evaluated	# seeds infected w/fungus	# seeds infected w/bacteria	total # seeds germinated
overall	659 (+ 660)[1]	361 (of 659)	23	23 (+ 45)
stratified[2] 10d	20	20	0	0
2wk	40	12	0	0
3wk	20	20	0	1 (died)
4 wk	35 (+ 15)	23 (of 35)	0	1 (died) (+ 2)
6wk	35	23	0	4
8wk	35 (+ 615)	24 (of 35)	0	0 (+ 33)
10wk	20	20	0	3
12wk	20	20	0	7
intact	330 (+ 620)	259 (of 330)	0	18 (+ 34)
no caruncle[3]	251 (+ 40)	103 (of 251)	18	3 (+ 11)

[1]. Numbers in parentheses (+ #) indicate individuals that were not evaluated for microbial infection prior to planting in the greenhouse.

[2]. Individuals were placed on moistened blotter paper in sterile petri dishes and stratified at 4°C for the time period indicated.

[3]. Includes both individuals with and without the non-radicle end clipped in addition to removal of the caruncle.

Seedling Establishment

Among seeds from the above germination trials which were planted in the greenhouse following various treatments, only 52 individuals (6%) germinated. Seedlings grew slowly following emergence, and most had only reached the 4 - 6 leaf stage 6 months after germination. During late summer, growth ceased, and by September individuals began to develop fall coloration, with leaves turning an orange-red color. All seedlings checked for root development had produced a taproot approximately 4 - 6 in in length. Regardless of whether germination occurred in the petri dish or in dibble tubes/pots in the greenhouse, the radicle emerged first and grew to approximately 4 - 6 in in length prior to emergence of the cotyledons. This process typically took 1 - 2 mo, except for individuals that had been stratified for 10 - 12 wk; in these individuals cotyledons emerged within 1 wk to 1 mo. Removal of the seedlings from the dibble tubes to observe root development did not appear to affect subsequent seedling growth and development.

NOTE: Seeds from several of the germination trials described above are beginning to emerge 6 - 12 mo after planting in the greenhouse. One intact, non-treated individual and 1 individual from which the caruncle was removed, with no further treatment, germinated 12 mo after planting in the greenhouse. In addition, 5 intact, non-treated seeds germinated 6 mo after planting in the greenhouse.

Fire Severity in "Croton Canyon"

The results of the on-site fire severity analysis are summarized in Table 4. The highest fire severity ratings were found on the hillsides while the lowest were found in the drainage bottoms. For transect #1, a band of intermediate ratings was found to exist on either side of the drainage bottoms. This band was only present on west facing aspects for transect #2.

The vegetative inventories revealed the dominant tree species in the area are Texas oak (*Quercus texana* Buckl.) and ashe juniper (*Juniperus ashei* Buchh.), while the understory included seedlings and saplings of the dominant trees along with several subordinate species such as deciduous holly (*Ilex decidua* Walt.), cat briar (*Smilax bona-nox* L.), and croton. Herbaceous species were also present but scarce.

Two distinct fuel beds were evident in "Croton Canyon", as well as in the areas where the fuel and vegetative inventories were taken. The structure and abundance of the fuels were found to vary by location within the canyon. The oak and juniper components were present on all sites. The drainage bottoms had relatively more deciduous Texas oak, and less shrubbery in the understory, and the hillsides had more evergreen juniper and more seedlings and saplings in the understory. Large dead fuels were rare on hillsides and abundant in drainage bottoms. This is probably related to the practice of juniper-post harvesting, which is common in both the vegetation plot sample areas and "Croton Canyon".

Table 4. Fire severity ratings for the 16 plots established in Croton Canyon.

Transect #	Plot #	Aspect	Slope	Location	Fire Severity Rating
1	1	East	10%	Drainage	L-1
1	2	None	0%	Drainage	L-1
1	3	West	5%	Drainage	L-1
1	4	West	20%	Hillside	L-2
1	5	West	30%	Hillside	L-3
1	6	West	40%	Hillside	M-4
1	7	West	10%	Hillside	M-5
2	8	East	10%	Hillside	M-5
2	9	Northwest	5%	Drainage	L-2
2	10	East	25%	Drainage	M-4
2	11	Southeast	10%	Hillside	M-5
2	12	Southeast	15%	Hillside	M-5
2	13	Southeast	40%	Hillside	M-5
2	14	Southeast	30%	Hillside	M-5
2	15	South	25%	Hillside	M-5
2	16	Southeast	10%	Hillside	M

Because of the differences found in the structure and loading of fuels within the canyons, two fuel models were created: one for hillsides and one for drainage bottoms. The basic characteristics of NFFL fuel model 9 were adjusted using the information from the vegetative inventories to create the two custom fuel models noted below. Note: 1hr fuels are those which are 0 to 1/4 inch in diameter, 10hr fuels range from 1/4 to 1 inch, and 100hr fuels range from 1 to 3 inches.

These custom fuel models were used in the TESTMODEL module of the BEHAVE fire behavior prediction system to simulate the fire behavior on both east and west facing aspects (Table 5). The fire burned through "Croton Canyon" on the 22nd of February. The values obtained from on-site weather data recorded for that day and used in the behavior simulation model are as follows:

20 foot Wind Speed = 20 mph
1hr fuel stick moisture = 2.9%
10hr fuel stick moisture = 3.0%
Temperature = 97° F
Relative Humidity = 11%

Winds were reported to be out of the east; however, given the shape of the local topography, wind direction was assumed to be blowing up-canyon. Because of this assumption,

Table 5. Summary of fire behavior simulations for the two custom fuel models for "Croton Canyon", Fort Hood Texas. Simulations were run for both east and west facing aspects.

	Drainage - East	Drainage - West	Hillside - East	Hillside - West
Rate of Spread (chains/hr)	4	4	175	174
Heat per Unit area (Btu/ft^2)	309	308	725	724
Fireline Intensity (Btu/ft/s)	22	22	2332	2308
Reaction Intensity (Btu/ft^2/min)	1613	1610	2523	2515
Flame Length (ft.)	2	2	16	16

the direction of maximum fire spread was calculated as being 45° (up-hill) from the direction of the wind vector, which ran northeast to southwest.

The fire behavior simulations agreed with the evidence from the fire severity plots. Fire behavior was highest on the hillsides and relatively low in the drainage bottoms. For those fire severity plots which could not be completed on-site because the trees were completely consumed, the flame length from the fire behavior simulations were used. All of these plots were on hillsides, thus, the calculated flame length of 16 feet places flame length within class #5 (Table 5). Although this is a very high flame length class, the eye-witness accounts reported much higher flame lengths, and the appraisals are probably an under-estimate of the actual flame lengths experienced on the hillsides.

The map in Figure 6 was created by extrapolating fire severity ratings found along the two transects. This map shows three different levels of fire severity. The majority of the area appears to have experienced a high upward heat pulse and a moderate downward pulse. The drainage bottoms experienced low upward and low downward heat pulses. A transition zone of intermediate fire intensities occurred between the drainages and hillsides. These areas typically experienced moderate upward heat pulses and low downward heat pulses. This band was found along the east side of the main draw where the fire backed down the east side of the drainage before making another uphill run towards the west. This pattern also agrees with wind direction and fire spread observations made by eye-witnesses.

Although formal post-fire regeneration evaluations will not begin until one year post-burn, the affected croton populations were evaluated in mid-October, several weeks after the return of normal rainfall patterns to the Fort Hood area. At that time, vigorous regrowth from basal sprouting was observed. No regrowth from above-ground branches was observed, even among individuals that were only slightly scorched.

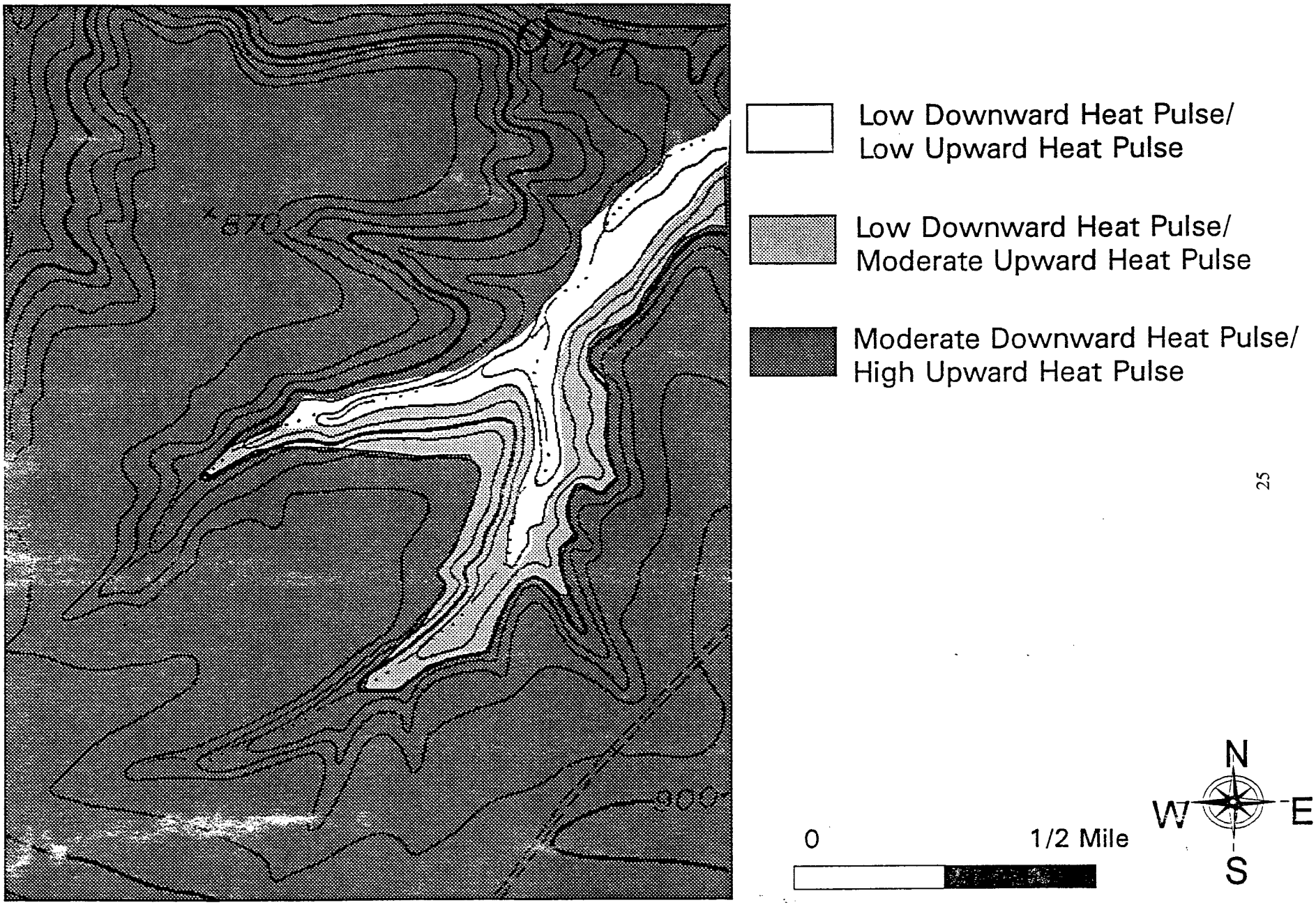

Figure 6. Zones of fire severity for the Croton Canyon Endangered Species habitat area.

Discussion

Germination

Despite observing close to 100% seed viability based on Tetrazolium staining analyses, seeds of *C. alabamensis* var. *texensis* showed very low levels of germination. In addition, germination was not enhanced by most of the standard methods utilized to overcome seed dormancy and stimulate germination. Failure to observe any germination among seeds from which the seed coat was removed nor from extracted embryos, regardless of treatment, suggests a possible stimulus for germination may be present in the seed coat. Failure to stimulate germination by treatment with KNO_3 suggests that dormancy of croton seeds is not the result of an osmotic imbalance that can be overcome by exposure to a salt treatment. Soaking seeds in GA_3 and exposing seeds to GA_3-moistened blotters both stimulated bacterial growth within the seeds and only slightly enhanced seed germination. Gibberellins are the family of growth regulators with the broadest spectrum of activity; however, whether the slight increase observed in germination was due to growth regulator action of GA_3 or to bacterial breakdown of the seed coat is unknown. Unfortunately, exposure to GA_3 also resulted in necrosis of both seed tissue and emerging radicles. It is possible that lower levels of GA_3 could be effective at stimulating germination without the corresponding tissue damaged observed at the 500 ppm level used in this study.

In this study, the only factor that significantly enhanced germination of croton seeds was an extended period of stratification at 4°C, lasting at least 8 wk. Germination rates increased with increasing length of the stratification period, up to 12 wk. This corresponds to the reproductive behavior observed in the field, whereby individuals germinate in early spring from seed shed the previous summer, and thus, pass through a period of chilling during the winter months. The need for a period of chilling for germination is an important factor for many species living where cold temperatures during the winter are unsuitable for seedling growth. Typically, the longer the period of stratification needed for germination, the deeper the dormancy of the seed (Bewley and Black 1985). A long prechilling requirement may also serve as a mechanism for preventing germination during brief warm spells in winter, which would be detrimental to seedling survival. Periodic mid-winter warm spells are a common occurrence in central Texas, and the requirement for extended stratification would prevent premature germination of croton seedlings during such periods. Imbibition of water by the seed is usually necessary before chilling is effective; thus, part of the pre-chilling period may be spent reaching adequate hydration (Bewley and Black 1985), and may also contribute to the long pre-chilling requirement for germination of croton seeds.

The development of microbial contamination does not appear to hinder germination of croton seeds, and may be beneficial. Among germinants from seeds which had been evaluated for microbial infection, 87% of the seeds were infected with fungal and/or bacterial contamination prior to germination. In those trials where microbial infection was not recorded for each individual, visual estimates of fungal contamination ranged from 70 - 100% of the seeds, regardless of treatment. In most cases, microbial infection began in the caruncle region, subsequently spreading to cover the entire seed. This suggests that the origin of infection was internal (endophytic). It is possible that a symbiotic relationship, with microorganisms sustained in the caruncle of the seeds and microbial activity stimulating breakdown of the seed coat and

subsequent germination, may play a role in preventing seeds from germinating during the hot, dry summer months immediately following seed maturation. With the onset of spring rains, the increased soil moisture levels combined with warmer temperatures could stimulate microbial activity in the caruncle region, ultimately leading to the breakdown of the seed coat, allowing germination to occur. A symbiotic relationship between microbial infection of the seed and germination could explain, in part, the absence of *C. alabamensis* var. *texensis* from apparently suitable habitats. If the microenvironment was not conducive to survival of the appropriate microorganism(s) needed to stimulate germination, seedling establishment would not be successful in that area.

Removal of the caruncle reduced fungal infection of the seed, but did not eliminate it. If the fungal strain observed growing on the seeds is endophytic, it is possible that it prefers the presence of the caruncle for optimal growth; however, it can develop in the absence of the caruncle, although growth is much slower. The observed development of bacterial infections only on seeds from which the caruncle had been removed is more likely related to external treatments (e.g., exposure to GA_3) than to presence or absence of the caruncle. Removal of the caruncle from the seed was also related to an observed decrease in germination, which may or may not be related to the corresponding decrease in fungal contamination. Although 3 intact seeds with no signs of microbial infection at planting were observed to germinate, it is quite possible these individuals developed fungal and/or bacterial infections in the soil prior to germination. The potential relationship between microbial breakdown of the seed coat and stimulation of germination did not become apparent until late in the germination trials. Additional studies carried out under controlled conditions are necessary before any conclusions can be drawn as to the role of microorganisms in the germination of croton seeds. If microbes are determined to stimulate germination, then it would be valuable to identify the specific organism(s) responsible and analyze the soil in regions with and without croton for presence or absence of the organism(s) involved.

The emergence of several individuals 6 - 12 mo. after planting in the greenhouse further suggests that croton seeds require a long period of after-ripening which is difficult to artificially overcome using standard dormancy-breaking techniques. While the highest levels of germination in this study occurred among intact seeds that had been stratified for several weeks and had also developed fungal infections prior to planting, the effects on germination of stratification and presence or absence of the caruncle are confounded. This is illustrated by the fact that 2 of the 3 germinants from intact seeds with no signs of fungal contamination at planting had been stratified at 4°C for 6 wk, and all 14 germinants from intact seeds that were infected prior to planting had also been stratified.

Seedling Establishment

The relatively long period of time between planting of the seed and emergence of the cotyledons through the soil appears to be associated with development of a taproot prior to seedling emergence. Such a pattern of development would be beneficial to the croton seedlings at Fort Hood, allowing for sufficient root development prior to the onset of dry conditions. Those individuals that were observed to germinate 6 - 12 mo after planting suggest that croton seeds produced in any given year also require different periods of after-ripening for germination

to occur. Unequal after-ripening ensures that all seeds from a given harvest do not germinate at the same time; therefore, if germination is followed by unsuitable conditions for seedling development, the entire seed lot will not be lost, as other individuals will germinate at a later date, once their after-ripening requirement has been met.

Fire Severity in "Croton Canyon"

Generally speaking, the fire which burned through portions of Fort Hood, including much of "Croton Canyon", was a fast-moving, high intensity fire. Eye-witnesses reported flame lengths on some portions of the fire in excess of 75 ft and rates of spread of up to 4 mph. Much of the fire behavior reported by eye-witnesses and recorded on video-tape can be classified as "extreme". According to eye-witnesses, the fire behaved as an independent crown fire for several hours during the first and second days of burning. The crown fire was driven by wind for most of its peak burning period; however, video-tape from the second day of burning and eye-witness descriptions indicate that the fire may have made the transition to plume-dominance. By mid-day on February 22nd, the convection column was towering 4,000 to 8,000 ft into the atmosphere and was only leaning over at about a 70° angle despite the 10 to 20 mph surface winds recorded. Firefighters reported a significant amount of spotting during this period and were surprised by the ability of the fire to spread rapidly downhill. Fire spread was also said to be "unpredictable" despite strong prevailing winds. All these clues suggest that at some point the energy from the fire probably overwhelmed the power of the wind, and for a short period of time the fire was essentially creating its own weather and dictating its own spread.

As is usually the case with any fire, the actual fire-effects were not uniform over the entire burned area. In "Croton Canyon", the differences between the fuels in the drainage bottoms and those on hillsides modified the fire behavior such that the severity of the fire in the drainage bottoms was less than that experienced on the hillsides. Besides the differences in the two fuel beds, the topography of the area may also have provided shelter from the wind in the drainage bottoms given that the prevailing wind was reported as coming out of the east. An east wind would have slowed as it crested and channeled up the canyon. By reducing the wind speed in the drainage bottoms, the resulting fire behavior was also reduced. The hillsides, meanwhile, were still exposed to the direct force of the wind. These areas would be expected to experience an increase in fire behavior as they would experience higher wind speeds than the drainage bottoms, especially from an easterly cross-wind.

The majority of the croton populations found in "Croton Canyon" were rooted on the banks of the drainage bottoms and almost all populations were found in the lowest fire severity class. Some individuals were consumed, but most did not experience direct flame contact. Temperatures from radiant and convective heat, however, were most likely high given the topography of the area and the amount of heat generated on the hillsides. All plants that did not sustain direct heat damage were undoubtedly stressed from the drying effect of the radiant and convective heat, as well as by the lack of significant rainfall both before and after the burn.

Croton reproduces by root-layering of prostrate branches, as well as by the production of viable seeds. The seeds require a stratification period during the winter, and scarification of the seed coat may enhance germination. With the relatively low downward heat pulses found in the drainage bottoms, the seed banks and root systems probably did not suffer irreparable damage.

Individuals that sustained significant damage to their above-ground plant parts should still be able to produce new basal sprouts. Informal observations made during October 1996, following a return to near-normal rainfall patterns at Fort Hood, noted vigorous regrowth from basal sprouting. The 6 - 8 in sprouts were most likely originating from the root system and were definitely not seedlings, as attempts to pull them up were unsuccessful. It is doubtful that seeds would germinate during the fall months; however, if the area experiences an adequate cold spell this winter, viable seeds may germinate in the spring. If seedling production is especially high in spring 1997, compared to previous years, this would indicate that fire is beneficial for seed germination through scarification of the seed coat, and/or for seedling establishment due to increased light reaching the developing seedlings. A more open canopy may also affect next year's flower and fruit production.

As ash material collects in the drainage bottom, more nutrients may become available. In addition, soil temperatures will become warmer as the darker surface increases the absorption of solar heat in the soil. These areas will also experience an increase in the amount of sunlight they receive because the canopy was opened-up by the fire. These factors should improve the growing conditions for the croton in the next few years, so long as the increased warmth and sunlight do not cause water availability to become limiting and the loss of vegetation from the hillsides doesn't greatly increase erosion. The removal of many large trees may increase future water availability in the drainage bottoms as less moisture is extracted from the soil by plants previously living on the hillsides.

Overall, the fire may have been to the crotons' advantage by reducing direct competition for resources by other species, setting the stage for a potential reproductive surge through basal sprouting and/or stimulation of seedling development, and potentially improving the growing conditions in and around the populations. The biggest concern at this point appears to be the need for adequate rainfall to relieve the plants from the stresses of the pre-fire drought, and further drying by the fire. Once an adequate amount of moisture becomes available, it seems feasible that the croton populations could fair well from this particular fire event.

References

Aplet, G.H., R.D. Laven, M.B. Falkner, and R.B. Shaw. 1994. Population and site characteristics of a recently discovered disjunct population of *Croton alabamensis* (Euphorbiaceae). Sida 16(1):37-55.

Brown, J. K., Oberheu, R. D., and Johnston, C. M. 1982. Handbook for inventorying surface fuels and biomass in the interior west. USDA Forest Service GTR INT-129, 48 p., illus.

Burgan, R. E. and Rothermel, R. C. 1984. BEHAVE: Fire behavior prediction and fuel modeling system - FUEL subsystem. USDA Forest Service GTR INT-167, 126 p., illus.

Bewley, J.D. and M. Black. 1985. Seeds: Physiology of Development and Germination. Plenum Press, New York.

Farmer, J.A. 1962. An ecological life history of *Croton alabamensis* E.A. Smith ex Chapman. Unpublished Ph.D. Dissertation, University of Alabama.

Farmer, J.A., and J.L. Thomas. 1969. Disjunction and endemism in *Croton alabamensis*. Rhodora 71:94-103.

Farnsworth, N.R., R.N. Blomster, and W.M. Messmer. 1969. A phytochemical and biological review of the genus *Croton*. Lloydia 32:1-28.

Ginzbarg, S. 1992. A new disjunct variety of *Croton alabamensis* (Euphorbiaceae) from Texas. Sida 15(1):41-52.

ISTA (International Seed Testing Association). 1985. Handbook on Tetrazolium Testing. ISTA, Zurich, Switzerland, 99 p.

Johnston, M.C. 1959. The Texas species of *Croton*. Southwest Naturalist 3:175-203.

McCaleb, N.L. 1985. Soil Survey of Coryell County, Texas. United States Department of Agriculture, Soil Conservation Service. United States Government Printing Office. Washington, D.C.

McDaniel, S. 1981. Status report on *Croton alabamensis* submitted to the United States Fish and Wildlife Service, Endangered Species Office, Region 4, Atlanta, Georgia.

Ryan, K. C. and Noste, N.V. 1984. Evaluating prescribed fires. In: Proceedings - Symposium and workshop on wilderness fire. USDA Forest Service GTR INT-182. pp 230-238.

Schotz, A. 1996. Alabama Natural Heritage Program. Pers. Comm.

Steed, D.L. 1993. Draft Report on Texabama *Croton* investigations, Fort Hood, Coryell and Bell Counties, Texas submitted by DLS Associates to the Directorate of Engineering and Housing Resource Management, Fort Hood, Texas, 31 p.

Van Wagner, C. E. 1973. Height of crown scorch in forest fires. Can. J. For. Res. 3(3):373-378.

Webster, G.L. 1993. A provisional synopsis of the sections of the genus *Croton* (Euphorbiaceae). Taxon 42:793-823.

Werchan, L.A., A. Lowther, and R. Ramsey. 1974. Soil Survey of Travis County, Texas. United States Department of Agriculture, Soil Conservation Service, in cooperation with the Texas Agricultural Experiment Station. Washington, D.C.

Evaluation of Genetic Diversity and Varietal Status of *Croton alabamensis* var. *texensis* at Fort Hood, Texas

Tracy Halward
Center for Ecological Management of Military Lands, Colorado State University

Introduction

Please refer to the preceding report "Germination and Seedling Establishment of *Croton alabamensis* var. *texensis* at Fort Hood, Texas" for a description of the species and its distribution.

This study was designed to evaluate relative levels of genetic diversity among and within the two known varieties of *C. alabamensis,* var. *alabamensis and* var. *texensis*. Individuals from several *C. alabamensis* var. *texensis* populations from both Fort Hood and Balcones National Wildlife Refuge in Texas, as well as individuals of *C. alabamensis* var. *alabamensis* from Bibb and Tuscaloosa counties, Alabama, are being analyzed to determine relative levels of genetic diversity among populations, as well as the taxonomic relationship between the two varieties. The results of our research efforts will be used to aid in the development of management plans for the species at Fort Hood.

Methodology

Populations Evaluated

Seeds of *Croton alabamensis* var. *texensis* were collected in May 1995 from all of the major known populations at Fort Hood, Texas and subjected to germination trials as reported above in "Germination and Seedling Establishment of *Croton alabamensis* var. *texensis* at Fort Hood, Texas". Seedlings from the germination trials were allowed to develop in the greenhouse for use in outplanting trials at Fort Hood and in preliminary genetic diversity evaluations.

Seeds of *Croton alabamensis* var. *alabamensis* from Bibb and Tuscaloosa Counties, Alabama were obtained from A. Schotz of the Alabama Natural Heritage Program, and seeds of *C. alabamensis* var. *texensis* from Balcones National Wildlife Refuge, Austin, Texas were obtained from C. Sexton of the US Fish and Wildlife Service. The seeds are being stratified at 4°C and will subsequently be planted in the greenhouse. DNA will then be extracted from fresh leaf tissue and analyzed for genetic diversity, as described below for the Fort Hood populations.

Genetic Diversity Evaluations

For each individual evaluated, DNA was extracted from fresh leaf tissue according to procedures adapted from Stewart and Via (1993) (Appendix A). Four individuals were chosen at random for primer screening. Random Amplified Polymorphic DNA (RAPD) analysis was performed according to procedures adapted from T. Lowrey (unpubl.) (Appendix B). To date, 18 primers have been evaluated.

Results

No variation was detected among individuals of *C. alabamensis* var. *texensis* with any of the primers evaluated to date.

Discussion

Although the RAPD results are still in the preliminary stages, failure to observe genetic variation among the individuals evaluated corresponds to the results from isozyme work by T. Lowrey (1995, unpubl. data). The isozyme study was conducted on a limited number of individuals, representing only 2 populations. The RAPD analysis reported here is still in the primer-screening stages and individuals from populations representing the entire diversity of locations at Fort Hood have not yet been evaluated. Therefore, no conclusions should be drawn from these preliminary results regarding genetic diversity within *C. alabamensis* var. *texensis*. In addition, seeds of *C. alabamensis* var. *texensis* from Travis county, Texas are still undergoing stratification and DNA extraction has not yet begun on these individuals. Once DNA extractions have been completed, RAPD analysis will be carried out to determine relative levels of genetic diversity among and within on-post and off-post populations. This will allow an evaluation of the relative importance of the Fort Hood populations to the recovery of this species, as compared to surrounding populations.

Seeds from *C. alabamensis* var. *alabamensis* populations are still undergoing stratification and have not yet germinated. Once these individuals have germinated and DNA has been extracted, a large set of informative primers will already have been identified from the above screening efforts, and genetic determination of varietal status for *C. alabamensis* var. *texensis* can begin.

References

Aplet, G.H., R.D. Laven, M.B. Falkner, and R.B. Shaw. 1994. Population and site characteristics of a recently discovered disjunct population of *Croton alabamensis* (Euphorbiaceae). Sida 16(1):37-55.

Farmer, J.A. 1962. An ecological life history of *Croton alabamensis* E.A. Smith ex Chapman. Unpublished Ph.D. Dissertation, University of Alabama.

Farmer, J.A., and J.L. Thomas. 1969. Disjunction and endemism in *Croton alabamensis*. Rhodora 71:94-103.

Farnsworth, N.R., R.N. Blomster, and W.M. Messmer. 1969. A phytochemical and biological review of the genus *Croton*. Lloydia 32:1-28.

Ginzbarg, S. 1992. A new disjunct variety of *Croton alabamensis* (Euphorbiaceae) from Texas. Sida 15(1):41-52.

ISTA (International Seed Testing Association). 1985. Handbook on Tetrazolium Testing. ISTA, Zurich, Switzerland, 99 p.

Lowrey, T. Professor of Botany, University of New Mexico. Unpubl. methodology.

Steed, D.L. 1993. Draft Report on Texabama *Croton* investigations, Fort Hood, Coryell and Bell Counties, Texas submitted by DLS Associates to the Directorate of Engineering and Housing Resource Management, Fort Hood, Texas, 31 p.

Stewart, C.N. and L.E. Via. 1993. A rapid CTAB DNA isolation technique useful for RAPD fingerprinting and other PCR applications. BioTechniques 14(5): 748-750.

Webster, G.L. 1993. A provisional synopsis of the sections of the genus *Croton* (Euphorbiaceae). Taxon 42:793-823.

Evaluation of Genetic Diversity Among and Within Populations of *Balduina atropurpurea* at Fort Stewart, Georgia

Tracy Halward
Center for Ecological Management of Military Lands, Colorado State University

Introduction

Balduina atropurpurea, a member of the Aster family (Asteraceae or Compositae), is an erect, perennial wetland herb. The taxa is considered a 'species of concern' (formerly, 3C) and is state listed in Georgia as rare (Smith 1994). The species typically occurs in wet areas such as peaty pitcher plant bogs, pine flatwoods, and pine savannas, all with seasonal standing water (Patrick 1994). It is most often associated with an understory of saw palmetto, shrub hypericum, and ericaceous shrubs (e.g., blueberry, huckleberry, staggerbrush, fetterbush, and dwarf kalmia) (Kral 1983). The species is a southeastern Coastal Plain endemic, thought to have originally been distributed from southeastern North Carolina to north-central South Carolina, south to central and south-central Georgia and into northeastern Florida, then west across northern Florida, through the panhandle, and into southeastern Alabama (Lutz 1995). Extant populations are known to occur in scattered locations in south to south-central Georgia, in northeastern Florida, and in southeastern Alabama. The largest, healthiest known populations of *B. atropurpurea* occur at Fort Stewart, Georgia. To date, a total of 20 populations have been identified at Fort Stewart. Populations range in size from < 10 to > 2000 individuals (Helton 1995).

Throughout its range, threats to the survival of the species include: hydrological disturbances; loss of habitat to agricultural, commercial, recreational, and residential development; and loss of habitat due to inappropriate site management, particularly fire suppression, which results in increased shading by shrubs and trees (Smith 1994). Military training exercises that either alter the hydrological regime, result in excessive soil disturbance, or suppress the occurrence of fire negatively impact *B. atropurpurea* populations on Fort Stewart. Several of the populations currently show significant impacts by tank maneuvers and/or off-road vehicle traffic (N2, N3, N6, N11, P1, P4), and many are in need of prescribed burning to reduce the encroachment of shrubs and woody vegetation, and to encourage the establishment of a healthy herbaceous layer (N4, N5, N6, N7, N8, N9, N13, N14, P3, P4, P5, P6). As of this writing, there are no existing management plans at Fort Stewart specifically designed for *B. atropurpurea*, and no U.S. Fish and Wildlife Service recovery plan has been prepared for the species.

Relatively little is known about the biology of *B. atropurpurea*. Individuals typically produce a rosette the first year, with inflorescences produced in the second and subsequent years. Parker and Jones (1975) reported that *B. atropurpurea* is self-incompatible, and that interspecific hybridization does not occur among species of *Balduina*. They also reported the occurrence of vegetative reproduction from root stocks. R. Determann of the Atlanta Botanical Garden successfully propagated seeds of *B. atropurpurea* following 4 weeks of cold stratification; the majority of the seeds germinated and produced robust rosettes (R. Determann, pers. comm. 1996). Investigations into the phenology, reproduction, seed dispersal, seedling establishment, and genetic diversity of *B. atropurpurea* are needed. Studies regarding the impacts of

hydrological and soil disturbances, as well as fire frequency and intensity, on the reproduction and health of this species are also necessary.

Evaluations of genetic diversity among and within populations of *B. atropurpurea* at Fort Stewart are vital for determining which populations, if any, contain unique genetic characteristics. If unique genetic populations are found, these should be given priority for conservation, as their destruction would lead to the loss of potential genetic diversity necessary for adaptation to environmental changes or habitat disturbance. Information regarding among- and within-population genetic diversity can be used during mitigation procedures for determining whether certain areas are of greater potential biological value than others, and should thus be protected from military training activities that could disturb the population or its surrounding habitat. Although it is only one piece of evidence, and other factors such as population health and community structure should be considered, documentation of genetic uniqueness would eliminate much of the guesswork involved in determining the biological value of a population and its resulting relative importance for conservation.

The objectives of this research were (1) to determine the relative levels of genetic diversity among and within a representative sample of the on-post populations of *B. atropurpurea*; and (2) to gain an understanding of the relationship between genetic diversity, morphological diversity, and habitat diversity. The information obtained from the study will aid in the development of a management plan for *B. atropurpurea* at Fort Stewart.

Methodology

Seedlings from 5 populations, representing the diversity of habitats in which *B. atropurpurea* is found at Fort Stewart, were obtained from R. Determann at the Atlanta Botanical Garden and transported to Colorado State University. The plants were transplanted into pots containing a commercial, soilless potting medium and placed in the greenhouse. Six individuals each from populations 1, 2, 3, and 4, and 4 individuals from population 5 were included in the analysis. DNA was extracted from fresh leaf tissues of each individual according to procedures adapted from Stewart and Via (1993) (Appendix A).

Random Amplified Polymorphic DNA (RAPD) analysis was conducted on the DNA extracts according to procedures adapted from T. Lowrey (unpubl.) (Appendix B). Four individuals were chosen at random for primer screening. Once a set of 30 informative primers was identified, RAPD analysis was conducted on the entire set of individuals described above. When RAPD analysis is complete, results from the genetic diversity evaluations will be correlated with the results from concurrent evaluations of habitat diversity among *B. atropurpurea* populations at Fort Stewart.

Results

Of the 28 primers evaluated to date, only 6 have revealed genetic differences among individuals. The differences have been randomly, and approximately equally, distributed among populations. Several primers have produced unscorable banding patterns due to high levels of polysaccharides complexed with the extracted DNA. So far, none of the cleaning efforts attempted have adequately removed the polysaccharides to allow for consistently clean amplification products. One primer (OPZ-6) produced a unique banding pattern for each

individual, indicating that the primer amplified a hypervariable region of DNA (e.g., a microsatellite) and therefore, could be useful for DNA fingerprinting of closely related *Balduina* genotypes.

Discussion

Based on the RAPD analysis results obtained thus far, it appears as though the populations at Fort Stewart are similar in genetic composition. The remaining 15 populations should be evaluated with a subset of the primers used in this preliminary study to determine whether any of the populations contain unique genetic characteristics, and thus deserve high priority for conservation efforts. This analysis would most likely be time-consuming and expensive, given the low levels of genetic variation detected among populations thus far. In addition, it is advisable to continue searching for a method which will adequately remove complexed polysaccharides from the extracted DNA samples before proceeding with RAPD analysis of the remaining populations. In the short term it might be more beneficial to conduct an in depth habitat characterization for each population and base conservation efforts on the uniqueness of habitats. Such a habitat characterization study has been planned, and should commence in spring 1997. However, genetic evaluations of all of the Fort Stewart populations should proceed concurrently with the habitat characterization evaluations. Long term survival of the populations is ultimately dependent on maintaining adequate genetic diversity among and within populations so as to allow for adaptation to environmental change and/or disturbance. Therefore, it is of vital importance to identify any populations that contain unique genetic characteristics, as these traits would be lost from the gene pool if the population(s) were eliminated. In addition, to obtain a better understanding of the relative value of the Fort Stewart populations compared to surrounding populations, habitat characteristics, population parameters, morphological diversity, and genetic diversity should be analyzed at several off-post populations and compared to the results from the on-post populations. A diverse range of populations should be sampled, including nearby off-post populations in Tattnall County, Georgia; as well as more distant populations known to exist in Georgia (Coffee, Long, Turner, and Jeff Davis counties), and in Nassau county, Florida. This would aid in the development of a recovery plan for the species within the state as a whole, in addition to the management plans developed for the species at Fort Stewart.

References

Helton, C. 1995. Final Report: *Balduina atropurpurea* Survey; Fort Stewart, Georgia.

Kral, R. 1983. A report on some rare, threatened, or endangered forest-related vascular plants of the South. USDA-Forest Service Technical Publication R8-TP2. U.S. Forest Service, Atlanta, Georgia.

Lowrey, T. Professor of Botany. University of New Mexico. Unpubl. methodology.

Lutz, K. 1995. Plant characterization abstract: *Balduina atropurpurea.* Draft Final Report of Fort Stewart Inventory. The Nature Conservancy.

Parker, E.S. and S.B. Jones. 1975. A systematic study of the genus *Balduina* (Compositae, Heliantheae). Brittonia 27(4):355-361.

Patrick, T. 1994. Personal communication with Inge Smith, North Carolina Natural Heritage Program, Raleigh, North Carolina, October, 1994. (see Smith, I.K., 1994, below)

Smith, I.K. 1994. Species stewardship summary: *Balduina atropurpurea*. Draft Element Stewardship Abstract for *Balduina atropurpurea*. North Carolina Natural Heritage Program, Raleigh, North Carolina.

Stewart, C.N. and L.E. Via. 1993. A rapid CTAB DNA isolation technique useful for RAPD fingerprinting and other PCR applications. BioTechniques 14(5): 748-750.

Evaluation of Genetic Diversity and Taxonomic Relationship Between *Silene hawaiiensis* and *Silene struthioloides* from the Island of Hawaii

Tracy Halward
Center for Ecological Management of Military Lands, Colorado State University

Introduction

Two species of *Silene*, *S. hawaiiensis* Sherff and *S. struthioloides* A. Gray, both endemic to Hawaii, have a long history of controversy surrounding their taxonomic relationship. Both species share similar morphological characteristics and their habitats overlap to some degree; however, each species possesses unique traits and occupies a different range of elevations as its primary habitat. Although they are currently classified as different species, the taxonomic relationship between *S. hawaiiensis* and *S. struthioloides* has long been a subject of debate. *S. hawaiiensis* is federally listed as threatened under the Endangered Species Act (ESA). Therefore, an understanding of the genetic relationship between this species and *S. struthioloides* is important for determining accurate population statistics for continued federal listing of *S. hawaiiensis*, as well as for development of an effective Endangered Species Management Plan (ESMP) for this species at Pohakuloa Training Area (PTA), a U.S. Army installation on the island of Hawaii.

S. hawaiiensis, a low sprawling to upright shrub, is found primarily in open dry areas on young ($\leq$ 4.0 ka) aa and pahoehoe lava flows, as well as on ash deposits, at 900 - 3000 m. Occasionally the taxa is found on older (10 - 20 ka) lava flows. Historically, the species is limited to the island of Hawaii in the Kilauea, North Kona, and Hamakua Districts, as well as along Saddle Road (Wagner et al. 1990). The current known distribution is limited to an area south of the Kilauea Caldera in Hawaii Volcanoes National Park, along the margins of the Mauna Kea and Mauna Loa Observatory Roads, as well as several populations on PTA (Shaw et al. 1993). *S. struthioloides*, a many-branched shrub is primarily found scattered in subalpine to alpine shrubland at 2150 - 3500 m. The species is known from both Maui and Hawaii (Wagner 1990).

Morphological comparisons based on dried herbarium specimens indicated no clear delineation between *S. hawaiiensis* and *S. struthioloides* (Popolizio et al. 1991, unpubl. data). These results are questionable due to the poor condition of many of the herbarium specimens evaluated and the possible mislabelling of many of the specimens (Popolizio 1995, pers. comm.). Biochemical analyses based on isozymes of 5 populations considered to be *S. struthioloides* from the Haleakala crater area and 3 populations considered to be *S. hawaiiensis* from Mauna Loa and Kilauea indicated a genetic distinction between the two groups (Westerbergh and Saura 1994). A dendogram constructed from unbiased genetic distances between populations showed the two species form separate clusters, and populations from specific regions clustered together within a species (Westerbergh and Saura 1994). The researchers concluded that very little gene flow was taking place among Hawaiian populations of *Silene*, and that the younger populations on the island of Hawaii were less polymorphic than those on the older island of Maui. They suggested that *S. hawaiiensis* was derived from *S. struthioloides* through founder effects followed by isolation, leading to genetic and morphological differentiation. They also indicated a need to

study *Silene* populations found between Mauna Kea and Mauna Loa to gain further insight into the possible derivation of *S. hawaiiensis*.

The purpose of this study was to use a more sensitive measure of genetic distinction (RAPD Analysis) than used in the past studies to evaluate the genetic relationship between *S. hawaiiensis* and *S. struthioloides*. The results will help settle the controversy over whether these are the same species (as suggested from morphological comparisons of herbarium specimens) or different species (as suggested from isozyme analysis of a limited number of individuals), and thus, aid in the development of an ESMP for *S. hawaiiensis* at PTA.

Methodology

Seeds from two populations of each species were planted in the greenhouse:

S. hawaiiensis: Siha-comp-1: greenhouse seed; harvested 5/94
Siha-comp-2: field seed from Puu Ahi; collected 11/92

S. struthioloides: Sist-comp-1: field seed from Mauna Kea; collected 11/92
Sist-comp-2: greenhouse seed; harvested 5/94

DNA was extracted from several individuals of each of the above populations, as well as several individuals of the related species, *S. lanceolata*, according to procedures adapted from Stewart and Via (1993) (Appendix A).

Random Amplified Polymorphic DNA (RAPD) analysis was conducted on the DNA extracts according to procedures adapted from T. Lowrey (unpubl.) (Appendix B).

Primers for RAPD analysis were screened on samples of 2 individuals each of *S. hawaiiensis* and the related but clearly distinct species, *S. lanceolata,* to identify informative primers. Informative primers were defined as those which produced clear, reproducible banding patterns showing differences between *S. hawaiiensis* and *S. lanceolata,* but not among individuals within each species. RAPD analysis was then carried out on DNA extracts from 7 individuals from Siha-comp-1, 8 individuals from Siha-comp-2, 6 individuals from Sist-comp-1, and 2 individuals from Sist-comp-2. Two DNA samples from *S. lanceolata* were included as checks. Once complete, results of the genetic diversity evaluations will be compared with the results from the morphological and biochemical diversity evaluations previously reported (Popolizio et al., 1991, unpubl. data; Westerbergh and Saura 1994, respectively).

Results

Of the 51 primers screened thus far, all but 3 showed genetic differences between *S. hawaiiensis* and *S. lanceolata*, and only 1 primer (OPZ-4) showed differences among individuals of *S. hawaiiensis*. This primer has not yet been evaluated on *S. struthioloides*. Of the 7 primers evaluated across all 3 species, 6 produced banding patterns that distinctly differentiated individuals of *S. hawaiiensis* from individuals of *S. struthioloides*. One of the primers produced similar banding patterns for individuals of *S. hawaiienis* and *S. struthioloides*. All primers evaluated produced very different banding patterns for individuals of *S. lanceolata* as compared to individuals of *S. hawaiiensis* or *S. struthioloides*.

Discussion

Although the results of this study should be considered preliminary, the relatively large amount of genetic variation observed between individuals of *S. hawaiiensis* and *S. struthioloides* as compared to the minimal levels of genetic variation detected among individuals within each of the species, suggests that genetic differentiation has taken place between these two species in Hawaii and supports the work of Westerbergh and Saura (1994). These results are also supported by the morphological differences observed in the field and the greenhouse, as well as differences in primary habitat, especially altitude, for each species. The results of the morphological comparisons based on dried herbarium specimens, which indicated no clear delineation between the two taxon, should be re-evaluated. Not only are closely related species often difficult to distinguish from each other based herbarium specimens, but the possible mislabelling of many of the herbarium specimens used in the initial evaluations of these two *Silene* species adds to the difficulty of obtaining reliable results from such comparisons (Popolizio 1995, pers. comm.).

Although no conclusions can yet be drawn from our results regarding species relationships between *S. hawaiiensis* and *S. struthioloides*, for now, the two species should be considered genetically distinct. In addition to the continuation of RAPD analysis for genetic diversity evaluations, there is a need for pollination/reproduction studies to examine interspecific compatibility. The information obtained from both genetic and reproductive studies is vital for determining accurate population size data and genetic purity for the continued listing of *S. hawaiiensis* under the ESA, as well as for the development of an ESMP for this species at PTA.

References

Lowrey, T. Professor of Botany. University of New Mexico. Unpubl. methodology.

Popolizio, C. et al. 1991 Unpubl. data

Shaw, R.B., Douglas, P.P., Castillo, J.M., Tierney, T.A., and Painter, E.L. 1993. Assessment of the Status and Recovery of Rare Plants in the Multipurpose Range Complex, Pohakuloa Training Area, Hawaii, Hawaii, Phase II, Complete Survey. Prepared for the U.S. Army Engineer Division Pacific Ocean, in Support of the Environmental Impact Statement for Operation of the Multipurpose Range Complex, Pohakuloa Training Area, Hawaii. Center for Ecological Management of Military Lands, Department of Range Science, Colorado State University, Fort Collins, CO.

Stewart, C.N. and L.E. Via. 1993. A rapid CTAB DNA isolation technique useful for RAPD fingerprinting and other PCR applications. BioTechniques 14(5): 748-750.

Wagner, W. L., Herbst, D. R. and Sohmer, S. H. 1990. Manual of the Flowering Plants of Hawaii. Bishop Museum Special Publication 83. University of Hawaii Press and Bishop Museum Press, Honolulu, HI.

Westerbergh and Saura. 1994. Genetic Differentiation in Endemic *Silene* (Caryophyllaceae) on the Hawaiian Islands. American Journal of Botany 81(11): 1487-1493.

Analysis of Genetic Variation in *Tetramolopium* species from Hawaii

Timothy Lowrey
Department of Biology, University of New Mexico

Introduction

The genus *Tetramolopium* in the Asteraceae presents an unrivaled opportunity to investigate genetic diversity in rare species because it has a wholly insular but disjunct distribution between the Hawaiian Islands and New Guinea. Additionally, 10 of the 11 Hawaiian species are considered threatened or endangered. The Pohakuloa Training Area (PTA) administered by the U.S. Army on the island of Hawaii is one of the most important areas of critical habitat for *Tetramolopium* in the Hawaiian Archipelago. Four species of *Tetramolopium* occur there Two of the rarest species of the genus and one variety of a third species are presently known only from PTA. The objective of this research was to sample and determine the amount of genetic variation that now resides in the populations of the Hawaiian species by analyzing morphological, enzyme, and DNA variation and comparing these data with the diversity that exists in the New Guinean species.

Methodology

Seeds and leaf tissues were sampled from most of the known populations of *Tetramolopium* on Hawaii, including all known populations of those species found at PTA (*T. humile* subsp. *humile* vars. *humile* and *glabrum*; *T. consanguineum*; *T. arenarium*; and *T.* sp. nov.). Leaf tissues and seeds were sampled from approximately 20 individuals per population. Leaf tissues were collected into vials and placed into liquid nitrogen or into packets of silica gel. Leaf tissue and seed samples were brought to the University of New Mexico. Plant material was processed for storage in an ultracold freezer, and seeds were planted in the greenhouse.

DNA was extracted from leaf tissue samples using similar procedures to those used at Colorado State University (Appendix A). RAPD analysis of the DNA extracts was according to similar procedures as those used at Colorado State University (Appendix B).

Results

The results from RAPD analysis were used to distinguish among Hawaiian species of *Tetramolopium*. One species previously unknown to science, *T. diersingii*, is being described.

Discussion

The genetic information obtained will be useful for biologists concerned with the preservation and management of rare species of *Tetramolopium* on PTA.

Analysis of Genetic Diversity in *Lepidium papilliferum* (Henders.) Nels. and Macbr. Using Isozyme and RAPD Data.

T. Lowrey
Department of Biology, University of New Mexico

Introduction

Lepidium papilliferum is a monocarpic annual/biennial endemic to the Snake River plains of Idaho. Populations occur in discontinuous depressions in sagebrush communities.

Methodology

Genetic diversity among populations from the U.S. Army Orchard Training Center in Idaho was evaluated using isozymes and RAPD markers. Ten isozyme loci and 36 RAPD loci amplified with 11 primers were included in the analysis.

Results

Isozyme banding patterns were indicative of diploid gene expression. Isozyme studies showed 33% of the loci were polymorphic with 3.3 alleles per polymorphic locus. The mean genetic identity between populations was 0.985. Analysis of RAPD variation showed 75% of the loci were polymorphic. Cluster analysis of genetic distances among individuals did not show significant population differences.

Discussion

Populations of *L. papilliferum* showed moderate levels of genetic variation and are not differentiated from one another, suggesting that there is significant gene flow among populations. Because initial investigations indicate that the populations of *L. papilliferum* at Orchard Training Center are genetically similar and represent the same species, the populations on the installation are not of as high a value as they would be if they were genetically dissimilar.

Comparison of Genetic Variation in *Carex inversa* from Hawaii and Australia

T. Lowrey
Department of Biology, University of New Mexico

Isozyme analysis of Hawaiian populations showed no genetic differences among individuals of *Carex inversa*. RAPDs, however, showed there was some genetic variability. The populations appear to consist of more than 1 clone in Hawaii. The variability is small and is consistent with a small number of individuals being established from a single introduction.

Genetic variation in *Rhododendron*

T. Lowrey
Department of Biology, University of New Mexico

RAPD analysis is incomplete due to difficulties with DNA extractions. A protocol developed for tropical trees has been recently received and is being tested on *Rhododendron*.

Evaluation of the Status of *Lipochaeta subcordata* and *Lipochaeta venosa* at the Pohakuloa Training Area and Nearby Areas of the Parker Ranch, Saddle Road Area, Hawai'i.

Sterling C. Keeley and Susan Garner
Department of Botany, University of Hawai'i

Introduction

The genus *Lipochaeta* (Asteraceae) is endemic to the Hawaiian Islands where it is called *nehe*. There are currently twenty recognized species (Wagner et al. 1990), half listed or proposed for Federal listing as rare and endangered. Species occur in a wide variety of habitats with several areas of potential or actual geographical overlap (sympatry). Morphological variability is substantial in the majority of species, but little is known of the genetic structure or population diversity underlying this variability. Taxonomic distinctions between morphologically similar species mean that clear species boundaries are uncertain for a number of species pairs.

Lipochaeta subcordata and *L. venosa* are two morphologically similar species whose relationship has been much debated (Wagner et al. 1986). These two species occur within a few kilometers of each other at the present time and may have grown adjacent to or with each other in the past, prior to separation by roads, grazing, and human activities. *Lipochaeta subcordata* is known from Bobcat Road and other areas of Pohakuloa Training Area (PTA), on the island of Hawai'i. The species is *not* considered to be endangered as it occurs elsewhere on the islands of Hawai'i and Kaua'i. However, the taxa is rated as a G-3 species by the Nature Conservancy. The taxa is very rare and local or, when wide-ranging, found only in a restricted range, making it vulnerable to extinction range wide. *Lipochaeta venosa*, is a Federally listed endangered species, occurring on a limited number of volcanic cones (pu'u) primarily on Parker Ranch lands near PTA.

The focus of this project was to assess the genetic variation in populations of both species using the molecular technique of RAPDs (Randomly Amplified Polymorphic DNA). Morphological variation is overlapping and the species may have been separated by picking the extremes of appearance and overlooking shared characteristics. Identifying evidence on shared and separate genetic variation is possible using the molecular technique of RAPDs. Additionally, the range of existing variation within populations, and where sufficient markers are present, may determine the existence of inbreeding or unequal gene exchange. The potential impact of this information on the status of rare and endangered species listings will be to confirm or show the lack of evidence to support existing, morphologically-based species designations. Should no detectable differences be found using RAPDs, a then widespread species might not be considered as endangered. If, on the other hand, the genetic data support prior morphologically-based conclusions that the species are separate entities, then continued preservation of the rare species would be warranted.

Methodology

RAPDs provide a technique for detecting population-level variation in DNA (Welsh and McClelland 1990, Arnold et al. 1991). This techniques allows a survey of numerous gene loci by detecting genes selected randomly from throughout the nuclear genome. A principal advantage

of the technique is that fine scale variations can be detected, while showing areas of relatedness. For example, two species within a genus can be distinguished, and at the same time evidence of common ancestry and, therefore, relatedness at the genetic level obtained.

RAPDs entail the use of the Polymerase Chain Reaction (PCR) that allows amplification (production of numerous copies) of the DNA fragments, through progressive cycles of DNA synthesis. Only if exactly the same DNA sequence is present in the genome will identical sequences be copied. If identical sequences are detected then the species are similar at that particular locus or loci. If there are differences in the DNA sequence then there will not be a match in the DNA synthesized during the RAPD reactions and differences will be detectable.

The DNA produced in the reaction is visualized using the process of electrophoretic separation on an agarose gel, staining with ethidium bromide, and visualizing the DNA fragments of different sizes with UV light. A photographs is taken as a permanent record. Marker DNA (lambda DNA digested to yield 1 kb size makers) is used to ascertain position and, therefore, homology of DNA segments produced by these reactions.

Sample Collection

Fresh plant material was collected from ten populations on the Big Island. Each collection was kept separate and drawings made of leaf outlines from a selected individuals. Six populations were collected at PTA, putatively all *Lipochaeta subcordata* (Figure XX). Four populations of *Lipochaeta venosa* were collected from Nohonohae and other nearby areas.

DNA Extractions

DNA was extracted separately and maintained as distinct samples from each plant in each population using the CTAB extraction procedure (Doyle and Doyle 1991). Large scale extractions (1.0-1.5 g fresh material) were purified using CsCl ultracentrifugation (55,000 rpm for 17-24 hr). DNA removed from the CsCl was washed to remove ethidium bromide and salts and was stored at -20°C until RAPD reactions were performed. Small scale extractions (<1 g) were also performed using a modified CTAB procedure for low volumes when material was limiting.

Results and Discussion

The meaning and extent of the differences observed is somewhat problematic because of the overall low levels of variation. A proportionally large number of primers have been surveyed as compared to mainland taxa. In any case, scorable differences were observed between the two species at a small number of loci. Also, there were common loci, supporting a common generic status. It is difficult to say with certainty if populations of either species are in Hardy-Weinberg Equilibrium (randomly breeding and suffering no inbreeding-depression) because of the kinds of loci available within each species. The lack of variable loci may be due to a bottleneck effect as populations are driven back to those few areas such as rock enclaves, where the pressures of grazing and fire are minimal. Historical records are limited, but indicate decreasing population sizes. The level of detectable variation with RAPDs is fine scale, and from throughout the entire nuclear genome. It is therefore more subtle than information obtained from allozyme surveys, secondary chemistry, and many other techniques.

If the variation seen to date is sufficient for species recognition, then the plants on Nohonohae and other pu'us near PTA are different species from those on PTA. The small, but perhaps critical differences, may be reason enough to continue separate species recognition in the face of nothing provides strong evidence against such recognition. Further, nowhere are *Lipochaeta* species common, even when they are not considered "rare" by Federal criteria. The G-3 designation applied even to *L. subcordata* by the Nature Conservancy states the species is vulnerable to extinction range wide, with fewer than 10,000 individuals remaining in <100 populations. Certainly there are not 100 populations remaining of either species, and their geographical extent even in Hawaii had decreased markedly from survey data collected by Cuddihy and others in 1983. *Lipochaeta venosa*, preserved in the enclosure on Nohonohae, has also diminished, even with protection. Resurveys for collections of living material undertaken in this study, show fewer individuals in smaller patches than recorded from the same areas on Nohonohae in 1983. From one pu'u to another the remaining populations of dozens to perhaps hundreds of individuals are so separated from one another as to constitute distinct and non-interbreeding entities. Field surveys indicate the species continue to be in jeopardy from fire and grazing activities.

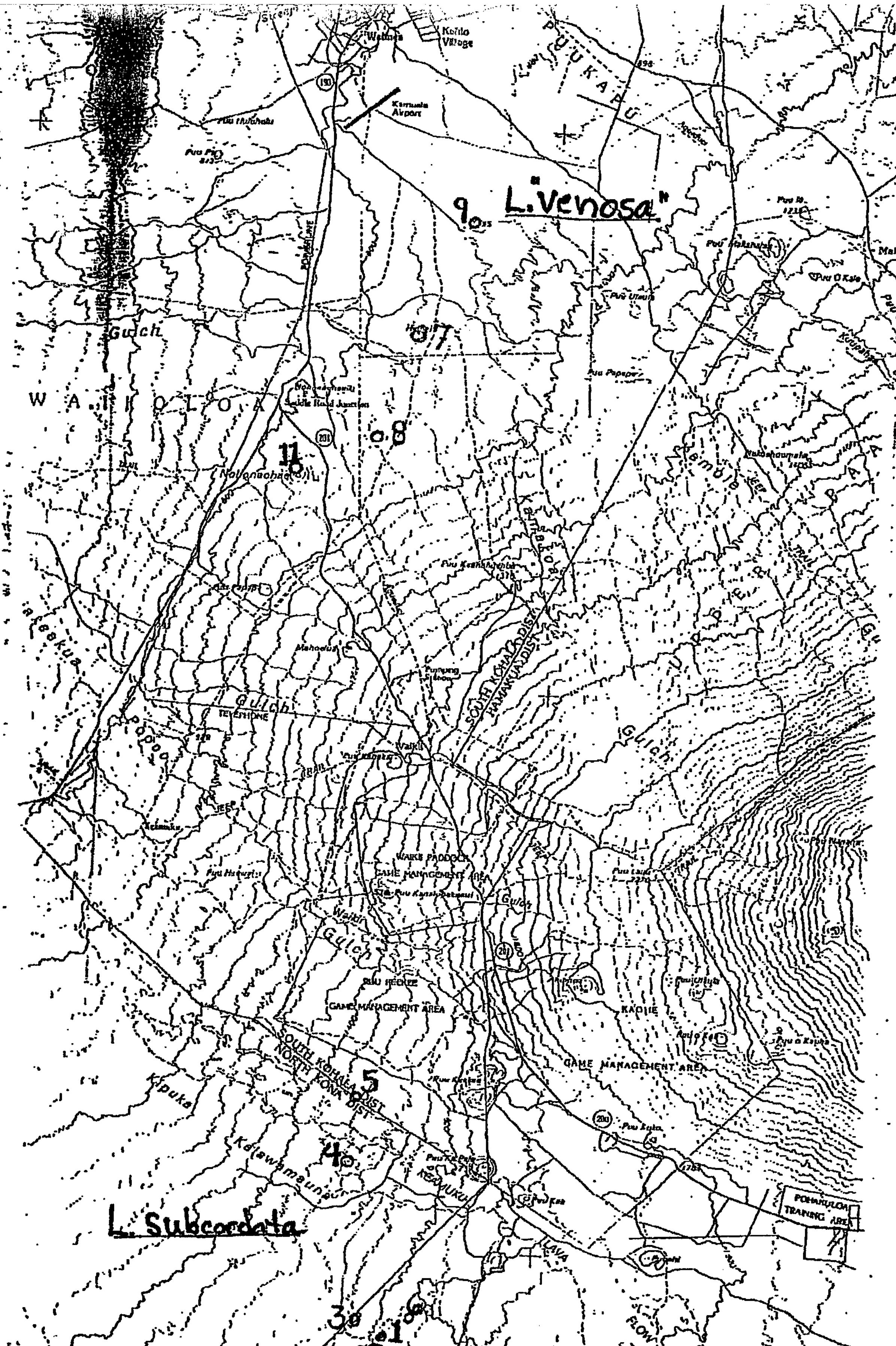
L. venosa
L. Subcordata
WAIKOLOA
Gulch
SOUTH KOHALA DIST.
NORTH KONA DIST.
HAMAKUA DIST.
GAME MANAGEMENT AREA
WAIKII PADDOCK
KAOHE
Waikii
Kipuka
Kalawamauna
POHAKULOA TRAINING AREA
LAVA
FLOW
9
7
8
11
5
4
3
1
2

Figure 1.

APPENDIX A

DNA Extraction Procedures Used for Genetic Diversity Evaluations of *Balduina atropurpurea*, *Croton alabamensis*, and *Silene* spp.

(adapted from Stewart and Via 1993)

For each sample, approximately 0.1 g of fresh leaf tissue was harvested and mechanically ground in an individual tissue grinder to which 5 ul B-mercapto-ethanol and 1 ml warm (65°C) CTAB extraction buffer (2% w/v CTAB, 1.42M NaCl, 20mM TRIS-HCl pH 8.0, 2 % w/v PVP and 5mM ascorbic acid) had been added, and the mixture was incubated in a 60°C water bath for 30 min. Each sample was then transferred to a clean eppendorf tube to which 500 ul chloroform:isoamyl alcohol (24:1) was added, and placed on a mechanical shaker at 5,000 rpm for 5 min., followed by centrifugation at 10,000 rpm for 5 min. The upper phase of each sample was transferred to a fresh eppendorf using a pasteur pipette and re-extracted with chloroform:isoamyl alcohol. The DNA was precipitated out of each sample by adding an equal volume of ice cold isopropanol to the tube and gently inverting the mixture. Samples were placed in a -20°C freezer overnight to further precipitate the DNA, then centrifuged at 5000 rpm for 5 min. The supernatant was discarded and the pellets cleaned of contaminating proteins by washing for 10 min. with 500 ul of a .2M sodium acetate/70% ethanol solution. The supernatant was poured off, and the pellets were air-dried and resuspended in 200 ul Tris-EDTA. Extracted DNA samples were stored at -20°C indefinitely. The quantity of DNA obtained per sample was measured using a spectrophotometer, based on the relative amounts of 260 and 280 nm wavelength UV radiation passing through the sample. The quality of the DNA extracted from each sample was determined by electrophoresing a subsample through a 1.0% agarose gel, staining the gel with ethidium bromide, and exposing the gel to UV light (ethidium bromide intercalates into the DNA molecule and fluoresces when exposed to UV light). This provided an estimate of the degree of degradation of the extracted DNA in each sample.

APPENDIX B

Procedure Used for RAPD Analysis of *Balduina atropurpurea*, *Croton alabamensis*, and *Silene* spp.

(after T. Lowrey, unpubl.)

Each RAPD reaction mixture was prepared by adding the following reagents to sterile microcentrifuge tubes: 18 ul sterile ddH_2O, 5 ul Master Mix [Electrophoresis Reaction Buffer; 10 mm each dATP, dCTP, dGTP, and dTTP; ddH_2O; and 2 mM $MgCl_2$ (magnesium chloride)], 1 ul primer (5 picamoles), and 1 ul DNA sample (diluted to between 5 and 50 ng/ul, depending on the species). The reaction mixture was gently vortexed, then centrifuged for a few seconds to collect the mixture at the bottom of the tube. Each reaction mixture was then overlain with 50 ul electrophoresis grade mineral oil to prevent evaporation during analysis. The reaction tubes were placed into individual wells, to which one drop of mineral oil had been added, in a DNA Thermal Cycler. The Thermal Cycler Program used was as follows: (Step 1) Hot Start of 2 min. @ 94°C; (Step 2) Addition of 0.5 unit Taq DNA polymerase to each reaction tube @ 80°C (held for 20 min.); (Step 3) Time Delay of 3 min. @ 94°C; (Step 4) 35 Cycles, each consisting of 1 min. @ 94°C (denaturing), 1 min. @ 38°C (first annealing), 30 sec. @ 54°C (second annealing), 2 min. @ 72°C (elongation); (Step 5) 15 min. @ 72°C (final elongation); and (Step 6) soak indefinitely @ 4°C. When the Thermal Cycler program was complete, 4 ul of electrophoresis tracking dye was added to each reaction tube, and the reactions were loaded into individual wells on 2.0% agarose gels. A molecular weight marker of known band sizes (100 base pair ladder) was included for band size comparisons with the reaction products. Samples were electrophoresed at 150 - 180 mA for 6 - 8 hr., stained with ethidium bromide for 30 - 60 min., destained with ddH_2O for 1 - 4 hr., and photographed over UV light. Variation in banding patterns among the DNA samples was analyzed from photographs of the gels using Polaroid 655 positive/negative film. Comparisons among and within populations were based on the presence or absence of specific bands produced during RAPD analysis.

Relevant Publications

Halward, T., T. Lowrey, K. Schulz, and R. Shaw. 1996. Germination requirements and genetic diversity in *Croton alabamensis* var. *texensis*. pp. 146-157, In: Proceedings of the Second Conference on Southwestern Rare and Endangered Plants, Sept. 11-14, 1995, Flagstaff, Arizona.

Halward, T., A. Hill, and R. Shaw. 1996. Evaluation of genetic diversity among and within populations of *Balduina atropurpurea*. (in prep.)

Halward, T. and R. Shaw. 1996. Evaluation of genetic diversity and taxonomic relationship between *Silene hawaiiensis* and *Silene struthioloides* from the island of Hawaii. (in prep.)

Halward, T. and R. Shaw. 1996. Evaluation of genetic diversity and varietal status of *Croton alabamensis* var. *texensis*. (in prep.)

Lowrey, T. 1996. Isozyme and RAPD variation in *Lepidium papilliferum* populations from Idaho. (in prep.)

Lowrey, T. 1996. Analysis of genetic variation in *Tetramolopium arenarium*: an endangered species from Hawaii. (in prep.)

Lowrey, T. 1996. *Tetramolopium diersingii*: a new species from Hawaii. (in prep.)

Lowrey, T. 1996. Comparison of genetic variation in *Carex inversa* from Hawaii and Australia. (in prep.)

Relevant Meeting Abstracts

Halward, T., T. Lowrey, K. Schulz, and R. Shaw. 1996. Germination requirements and genetic diversity in *Croton albamensis* var. *texensis*. Second Southwestern Rare and Endangered Plants Conference, Sept. 11-14, 1995, Flagstaff, Arizona.

List of Personnel Receiving Pay from this Effort

Colorado State University:	Robert Shaw, Director, CEMML Tracy Halward, Research Associate Patsy Douglas, " " Christopher Nelson, Temp. Hourly Kristina Vinsonhaler, " " Pam Northy, Account Tech I Rose Perez, Account Tech III Ernie Solano, Admin. Assist. I Darlene Maki, Admin. Assist. I Diana Czaja, Admin. Assist. II
University of New Mexico:	Tim Lowrey, Faculty (did not directly receive pay) Dixie Daniels, Temp. Hourly Ken Sylvestor, " " J. Weaver, " " Kimberly Taugher, " " C.K. Mazier, " " C.D. Saavedra, " " D.K. Davenport, " "
University of Hawaii:	Sterling Keeley, Faculty (did not directly receive pay) Susan Garner, Temp. Hourly C.Y. Kato, " "